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Elimination of extrachromosomally amplified *MYC* genes from human tumor cells reduces their tumorigenicity

(hydroxyurea/gene amplification/oncogenes/double-minute chromosomes)

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ABSTRACT **Oncogene amplification has been observed in a broad spectrum of human tumors and has been associated with a poor prognosis for patients with several different types of malignancies. Importantly, at biopsy, the amplified genes localize to acentric extrachromosomal elements such as double-minute chromosomes (DMs) in the vast majority of cases. We show here that treatment of several human tumor cell lines with low concentrations of hydroxyurea accelerates the loss of their extrachromosomally amplified oncogenes. The decreases in MYC copy number in a human tumor cell line correlated with a dramatic reduction in cloning efficiency in soft agar and tumorigenicity in nude mice. No effect on gene copy number or tumorigenicity was observed for a closely related cell line containing the same number of chromosomally amplified *MYC* genes. One step involved in the accelerated loss of extrachromosomal elements is shown to involve their preferential entrapment of DMs within micronuclei. The data suggest that agents that accelerate the loss of extrachromosomally amplified genes could provide valuable tools for moderating the growth of a large number of human neoplasms.**

Gene amplification produces multiple copies of genomic regions ranging from 100 kilobase pairs (kbp) to $>10^4$ kbp (see ref. 1 for review and references) and results in overproduction of proteins encoded by the genes within the amplified region. Since amplification of genes such as *NMYC*, *Her2/neu*, and *MYC* has been correlated with a poor prognosis (2–5), it is reasonable to infer that the overexpression of such genes contributes to tumor cell growth or survival. Furthermore, while amplification of a specific gene such as *Her2/neu* occurs in only 25% of human breast cancer (3–5), the total incidence of amplification in breast cancer exceeds 70% when those tumors with *MYC* (5), *hst-int-PRAD1* (6, 7), and *bcl1* amplification are included. If overexpression of each of these genes contributes to tumorigenicity, then strategies to decrease their expression may retard tumor growth. Consistent with this idea, experiments in which antisense RNA technology is used indicate that reducing oncogene expression slows tumor cell proliferation (8).

An alternative approach to moderate the growth of tumor cells containing amplified oncogenes is to remove the additional gene copies. It is striking that, at biopsy, amplified genes in human tumors almost always localize to acentric, heterogeneously sized, paired chromatin bodies called double-minute chromosomes (DMs; ref. 9; see ref. 10 for review). Therefore, we focused on strategies to eliminate DMs from cancer cells to effect the loss of the putative growth-augmenting genes they may encode. We concentrated on approaches that do not require knowledge of the genes contained within the DMs, or of the sequences required for DM replication, to attempt to provide generally useful elimination therapies that could be implemented with minimal delay. A particularly promising protocol involves treatment with low concentrations of hydroxyurea (HU), since this drug has been shown to increase the rate at which DMs containing a variety of drug-resistance genes are lost from mouse (11) and human tumor cell lines (12). Since the DMs in these studies were very different in size, and were contained in the nuclei of highly diverged species, the data strongly indicate that the mechanism by which HU accelerates DM loss is not likely to be restricted to particular genes or cell types.

The present study demonstrates that HU increases the rate at which extrachromosomally amplified oncogenes are lost from four different human tumor cell lines. The data reveal that reducing the copy number of *MYC* genes correlates with substantial decreases in the cloning efficiency of a human carcinoma cell line in soft agar and tumorigenicity of treated cells in a nude mouse model system. The mechanism by which HU affects DM loss is shown to involve the preferential capture of DMs within micronuclei.

METHODS

Cell Lines. An early passage (passage 46) of the HL60 promyelocytic leukemia cell line was obtained from S. Collins (Fred Hutchinson Cancer Center). This cell line contains 16–32 copies of the *MYC* oncogene, the majority of which localize to extrachromosomal molecules ranging from 250-kbp episomes to DMs (13, 14). Passage 67 subclone 173 contains a median of 8 DMs per cell and was used for this study (14, 15). A previously described subclone of COLO 320DM (American Type Culture Collection; ref. 16), which contains a median of 30 DMs per cell and an amplicon of 120–160 kbp (14), was used. The COLO 320HSR (HSR, homogeneously staining region) line has approximately the same number of copies of *MYC* dispersed at several chromosomal sites (ref. 14; this study). The NB4 neuroblastoma cell line (passage 20; kindly provided by J. Casper and V. Piaskowski, Milwaukee Children Hospital) has an ≈50-fold amplification of the *NMYC* gene localizing to ≈1000-kbp episomes and DMs (17). The SF188 glioblastoma multiform cell line, passage 220, has a 25-fold amplification of the *MYC* gene localizing to heterogeneously sized episomes, with a

Abbreviations: DM, double-minute chromosome; HU, hydroxyurea; FISH, fluorescent *in situ* hybridization; HSR, homogeneously staining region.

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minimum size of 100 kbp (D.D.V.H., unpublished data), and DMs (18).

The HL60 and COLO 320 cell lines were grown in RPMI 1640 medium with 10% fetal bovine serum (FBS), and NB4 and SF188 in RPMI 1640 medium containing 20% FBS and 2 mM glutamine. Based on previous work by Snapka and Varshavsky (11), HU (Squibb) was added on day 0 of culture at the concentrations indicated and was replaced each time the cells were passaged. All cells were passaged by a 1:10 dilution of confluent cultures every 3–7 days. Cell growth was determined with a hemocytometer.

Tumorigenicity Assays. Cells exposed to various concentrations of HU for 21 days *in vitro* were washed, grown without HU for 48 hr, and then plated at a concentration of 2500 cells per 35-mm Petri dish by a soft agar cloning technique (19). The number of colonies containing ≥50 cells was determined 21 days later. Alternatively, 5×10^6 cells treated with HU as described above were injected into the suprascapular region of *nu/nu* BALB/c mice (Charles River Breeding Laboratories) in groups of 8. The mice were kept in a germ-free laboratory according to U.S. Department of Agriculture guidelines, and they were observed twice weekly for tumor formation. The volume of each tumor developing in the suprascapular area was determined by the formula volume = $(W^2 \times L)/2$ (W, width; L, length).

Evaluation of Cells for Micronuclei and Localization of *MYC* Genes. Micronuclei were scored in preparations of metaphase chromosome spreads and interphase nuclei (15, 20). The cells were exposed to Colcemid (0.1 μg/ml; GIBCO) for 1–3 hr, incubated in 0.075 M KCl for 20 min, fixed in methanol/glacial acetic acid (3:1), and dropped on wet slides. Portions of tumors that had been established *in vivo* were either used immediately for preparation of metaphase spreads or reestablished in cell culture to enable a comparison of the number of *MYC* DMs per cell under various growth conditions.

The *MYC* cosmid (Yuxin Yin, Salk Institute) and centromere probes (Oncor, Gaithersburg, MD) used for *in situ* hybridization were labeled with biotin-16-dUTP (Boehringer Mannheim) by nick-translation with a reaction mixture containing all four dNTPs (Pharmacia). Fluorescent *in situ* hybridization (FISH) was conducted as described by Pinkel *et al.* (21).

RESULTS

HU Accelerates the Loss of Extrachromosomal Oncogenes. Since HU has been shown to accelerate the rate of elimination of extrachromosomally amplified drug-resistance genes (11, 12), we tested its efficacy for reducing oncogene copy number. HU treatment caused a substantial reduction in the copy number of extrachromosomally amplified oncogenes in every cell line analyzed (see Fig. 1*A* for one example), but the rates varied for each. For example, while >50% of the amplified genes were eliminated from HL60 and NB4 cells at 50–100 μM HU after 3–6 cell doublings, only a 30% loss was achieved in the same number of cell doublings in SF188 cells exposed to 150 μM HU. Furthermore, HU accelerated the loss of circular molecules ranging in size from 120-kbp episomes to multimegabase DMs (data not shown). The effects of HU were specific for extrachromosomal structures, since application of the same treatment strategy to COLO 320HSR cells containing chromosomally amplified *MYC* genes did not produce a significant reduction of oncogene copy number (Fig. 1*B*).

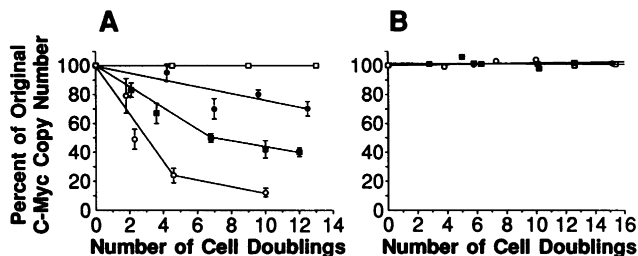

FIG. 1. HU accelerates selective elimination of extrachromosomally amplified genes. COLO 320DM (*A*) and COLO 320HSR (*B*) cells were treated with 0 (□), 50 (●), 100 (■), or 150 (○) μM HU for the indicated number of cell doublings. DNA was prepared and the fraction of the initial *MYC* copy number was determined by dot blotting and hybridization to a *MYC* cDNA probe (Oncor). The amount of DNA loaded onto each sample was determined by hybridization with a probe for rRNA-encoding DNA.

Loss of Amplified Oncogenes Is Associated with Reduced Tumorigenicity. The HU treatment protocol described above eliminated up to 90% of the *MYC* genes from COLO 320DM cells, but it was unclear whether loss of this number of *MYC* genes would reduce tumorigenicity. We explored this issue by first measuring the ability of the cells to form colonies in semisolid medium since anchorage-independent growth has long been used as one indicator of tumorigenicity (22). COLO 320DM and COLO 320HSR cells were treated with various

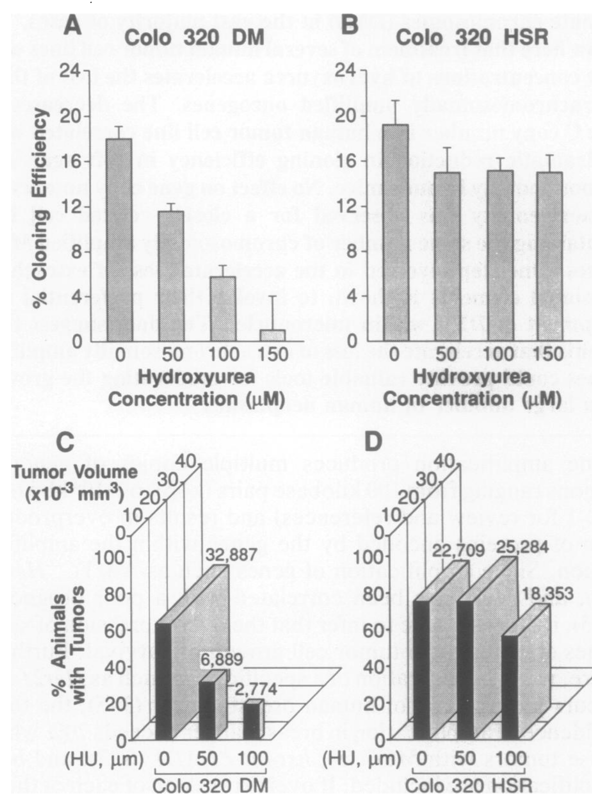

FIG. 2. Reducing *MYC* copy number decreases tumorigenicity of COLO 320DM cells. COLO 320DM (*A* and *C*) and COLO 320HSR (*B* and *D*) cells were treated with HU at the indicated concentrations for 21 days, commencing on day 0, with fresh HU being added at each passage. After 21 days of exposure, cells underwent the following number of population doublings (pd): 0 HU, 13 pd; 50 μM HU, 12.5 pd; 100 μM HU, 12 pd; 150 μM HU, 10 pd. Plating efficiency in soft agar was determined after washing the cells to remove HU (*A* and *B*). Identically treated cells were also injected into the suprascapular region of BALB/c *nu/nu* mice (5×10^6 cells per mouse; eight mice per data point) and were allowed to grow for ≈6 weeks. The number of animals with tumors and the volume of the tumors were determined. χ^2 analysis revealed a significant difference between the fraction of untreated animals developing tumors when injected with untreated COLO 320DM and those injected with COLO 320DM cells treated with 100 μM HU ($P < 0.02$), and an analysis of variance revealed a significant difference in the volumes of the tumors detected ($P = 0.01$). No significant difference for either parameter was noted between the control and treated COLO 320HSR cells.

concentrations of HU, and their cloning efficiency in soft agar was determined. Fig. 2*A* shows that there is a dose-dependent reduction in the cloning efficiency of COLO 320DM but not of COLO 320HSR cells (Fig. 2*B*), which parallels the decrease in *MYC* copy number reported in Fig. 1*A*. The lack of effect of HU on the cloning efficiency of COLO 320HSR cells indicates that the profound effects of the drug on COLO 320DM cells cannot be explained by nonspecific cytotoxicity or by inhibition of *MYC* activity.

A more direct indicator of tumorigenicity is to determine the ability of the cells to form a tumor in nude mice (23). We first gained insight into whether elevated *MYC* copy number

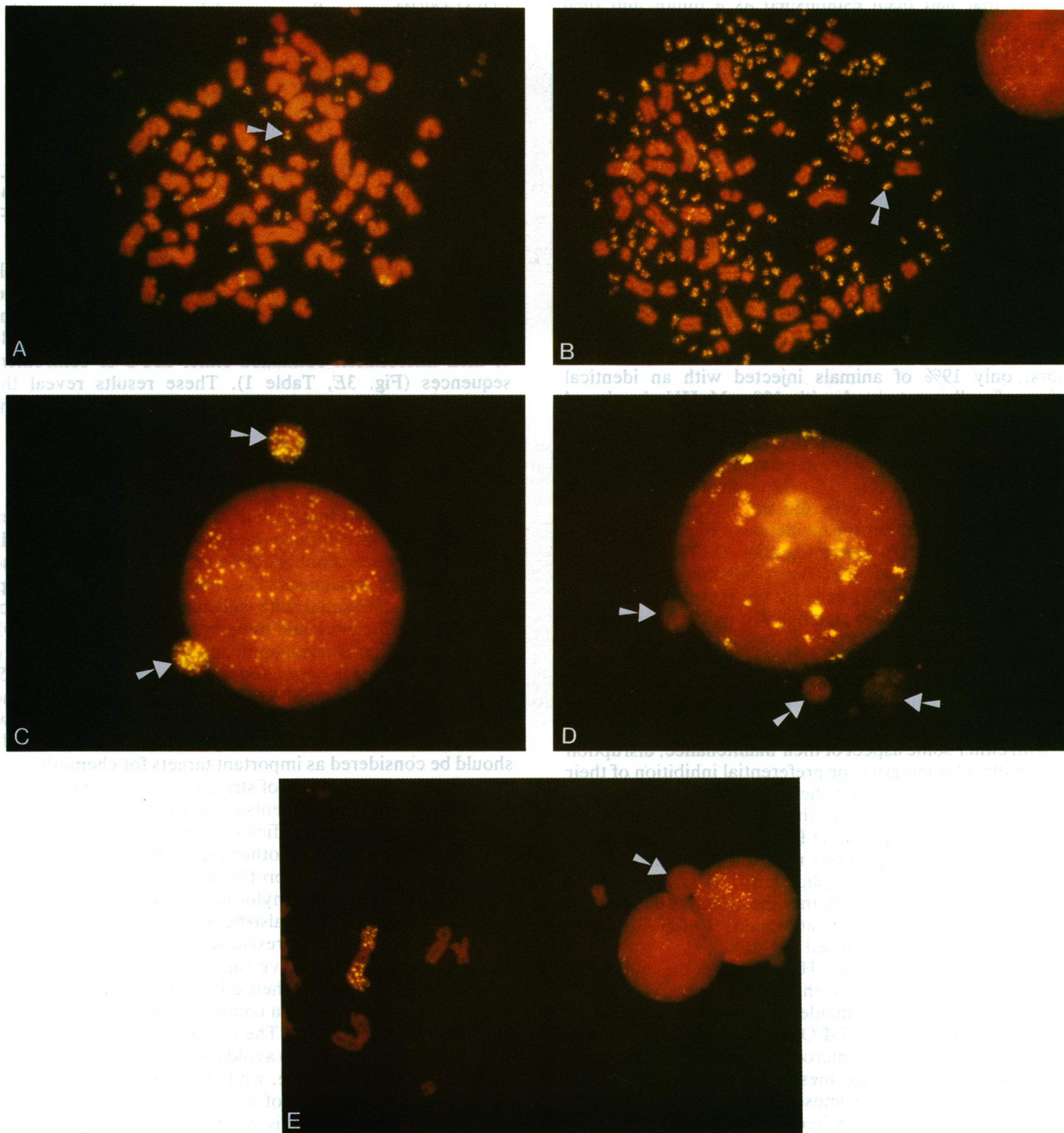

FIG. 3. Mechanism of selective elimination of extrachromosomally amplified *MYC* genes involves entrapment by micronuclei. FISH using a *MYC* cosmid probe was performed on metaphase spreads prepared from COLO 320DM cells grown in cell culture (*A*) or established as a nude mouse tumor for 8 weeks and analyzed within 24 hr of removal (*B*). Note that the sizes of the DMs are approximately the same for both growth conditions, but the number of DMs is 3- to 4-fold higher in cells propagated as a tumor *in vivo* (see text; arrows, typical *MYC* DMs). (*C*) COLO 320DM cells treated with 100 μM HU for 7 days [≈4 population doublings (pd)] were analyzed by FISH with a *MYC* cosmid. Arrows denote two micronuclei, which are heavily labeled with the probe. (*D*) Fraction of the same preparation shown in *C* was hybridized with a centromere cocktail probe, which detects most human chromosomes. Note the substantial hybridization within the large nucleus and the lack of hybridization within the three micronuclei (arrows). (*E*) COLO 320HSR cells were treated with 150 μM HU for 21 days (≈10 pd). Cells obtained from a metaphase preparation made at this time were hybridized with the fluoresceinated *MYC* cosmid. Note hybridization within the large nucleus and lack of hybridization within the micronucleus (arrow). A chromosome that hybridizes with the *MYC* cosmid was detected on a metaphase plate adjacent to the nuclei shown and represents the predominant site of intrachromosomal amplification of *MYC* genes in these COLO 320HSR cells.

might contribute to tumor growth *in vivo* by comparing the *MYC* DM content of cells propagated in cell culture relative to that of tumors established from COLO 320DM cells in nude mice. COLO 320DM cells contained an average of 28 DMs per cell, while tumor cells analyzed directly after explanation had an average of 95 DMs per cell (Fig. 3 *A* and *B*). Cells that had been established as a tumor and then returned to culture for 3 weeks showed a reduction from 95 to 28 DMs per cell. These changes in DM number were highly significant ($P < 0.0001$; Wilcoxan or Kruskall–Wallis tests). These data strongly suggest that additional *MYC* copies contribute to tumor growth or viability *in vivo*.

The experiments described above indicated that the magnitude of the reduction of *MYC* copy number in COLO 320DM cells achievable by HU treatment might reduce their tumorigenicity in nude mice. Therefore, COLO 320DM cells treated *in vitro* with HU as described above were injected into the suprascapular region of nude mice, and the incidence and volumes of the tumors formed were determined. Fig. 2*C* shows that *in vitro* pretreatment of COLO 320DM cells with HU substantially reduced the ability of the COLO 320DM cells to form tumors in animals. While 63% of animals injected with cells that were not pretreated with HU formed tumors, only 19% of animals injected with an identical number of cells pretreated with 100 μM HU developed tumors. Importantly, the tumors that developed with the HU-treated cells were <1/10th the volume and took longer to appear than those that were generated by the injection of untreated control cells (Fig. 2*C*). In contrast, HU pretreatment of COLO 320HSR cells did not have a statistically significant effect on either the incidence of tumors formed or their volumes (Fig. 2*D*). Taken together, the results demonstrate that reduction in *MYC* copy number that can be achieved in COLO 320DM cells by the HU treatment conditions used is sufficient to significantly reduce their tumorigenicity.

Mechanism of HU Selectivity Involves Preferential Entrapment of Extrachromosomally Amplified Genes in Micronuclei. HU could potentially accelerate the loss of extrachromosomal elements by several mechanisms including interference with either some aspect of their maintenance, disruption of their molecular integrity, or preferential inhibition of their replication. HU did not affect the structure of the submicroscopic circular precursors of the *MYC* DMs (i.e., *MYC* episomes; ref. 14) (B.J.F. and D.D.V.H., unpublished data), and the low HU concentrations used did not affect cellular DNA synthesis or episome replication (12). These negative results led us to investigate whether the selective elimination of extrachromosomal elements at low doses of HU is mediated by micronuclei as proposed (24–26).

We first determined whether HU increases the number of micronuclei formed. Table 1 shows that there is a dose-dependent increase in the incidence of micronucleation in both COLO 320DM and COLO 320HSR cells. Since the HU-dependent increase in micronuclei formation is quantitatively indistinguishable for these two cell lines, the selectivity of HU for extrachromosomal elements cannot be attributed to micronucleation frequency alone.

We next used FISH with probes that detect either *MYC* or centromeric repetitive sequences to investigate whether micronuclei preferentially capture small and/or acentric structures such as DMs. Typical results of such experiments are shown in Fig. 3*C* and are quantitated in Table 1. Hybridization of COLO 320DM cells with a biotinylated probe revealed intense staining of the majority of DMs in metaphase preparations (Fig. 3*A*). Fig. 3*C* and Table 1 show that an average of 68% of the micronuclei derived from COLO 320DM cells contain numerous *MYC* sequences. By contrast, hybridization of micronuclei in the same preparation with a centromere "cocktail" probe, which detects most human chromosomes,

Table 1. Preferential entrapment of extrachromosomal elements by micronuclei

Cell line	HU, μM	Micronuclei, %	% hybridizing	
			With *MYC*	With centromere
COLO 320DM	0	4.4	50.0	7.4
	50	5.1	68.6	3.7
	100	8.4	80.0	4.8
	150	17.1	73.7	6.7
COLO 320HSR	0	4.4	8.3	12.5
	50	6.2	3.0	5.9
	100	8.8	17.6	1.1
	150	11.8	3.3	10.9

The indicated cell lines were treated with HU for 7 days and then prepared for *in situ* hybridization. Each data point derives from counting ≈1000 nuclei.

revealed that an average of only 6% of the micronuclei contain centromeric sequences (Fig. 3*D*, Table 1). When COLO 320HSR cells were treated with HU under the same conditions and were analyzed identically, an average of 8% of their micronuclei contained either MYC or centromeric sequences (Fig. 3*E*, Table 1). These results reveal that micronuclei preferentially entrap acentric extrachromosomal DNA molecules.

DISCUSSION

These studies show that chronic treatment with low concentrations of HU accelerates the loss of extrachromosomally amplified oncogenes but has little or no effect on chromosomally amplified oncogenes. The reduction in oncogene copy number was correlated with substantial reductions in soft agar cloning efficiency and tumorigenicity of COLO 320DM cells in nude mice. Since amplification and overexpression of certain oncogenes is correlated with a poor prognosis (2–5), and the majority of amplified genes localize to extrachromosomal elements in human cancers analyzed at biopsy (10), we propose that DMs, and not the specific genes they encode, should be considered as important targets for chemotherapy.

The potential usefulness of strategies to accelerate the loss of extrachromosomal elements encourages a search for other agents that may be more efficient than HU or that potentiate its effects. Guanazole, another inhibitor of ribonucleotide reductase, etoposide, and proflavine (inhibitors of topoisomerase II), and difluoromethylornithine (an inhibitor of ornithine decarboxylase) have also been reported to promote loss of unstably amplified drug-resistance genes or oncogenes (27, 28). Since these agents have rather diverse mechanisms of action, we speculate that their effects on extrachromosomal elements may derive from a common ability to stimulate the formation of micronuclei. The concentration of each agent must be chosen carefully to avoid paradoxical effects on gene amplification. For example, while HU concentrations < 200 μM stimulate elimination of extrachromosomally amplified DNA (refs. 11 and 12; this report), higher concentrations appear to increase amplification frequency (29). The mechanism for the latter effect may involve chromosome breakage mediated by HU-induced inhibition of DNA synthesis (30, 31). We did not explore the effects of HU concentrations exceeding 150 μM because of the substantial growth rate reductions produced at such drug levels.

The extremely high sensitivity and resolution of the FISH technique provides convincing evidence that micronuclei preferentially capture extrachromosomal molecules and only infrequently contain chromosomes. The frequency of micronuclei containing chromosomes with homogeneously staining regions was too low to reduce *MYC* copy number

sufficiently to register in either DNA blotting analyses or in any of the biological tests we have used. However, other reports show that treatment of cells with high concentrations of clastogenic agents results in a substantial fraction of micronuclei containing centromeric sequences (32, 33). In light of our results, it is likely that such treatments fragmented the chromosomes to a size that could be captured within the micronuclei. If this interpretation is correct, then size, and not the presence or absence of a centromere, may be the major determinant of what can be trapped within micronuclei. The mechanism by which DMs are lost from the cell once they are captured within micronuclei remains to be elucidated. It is possible that the micronuclei are lost to the cytoplasm and are inefficiently reincorporated into the nucleus at mitosis. Alternatively, micronuclei may contain nucleases that degrade the chromatin within them. Experiments to differentiate among these alternatives remain to be done.

Our studies revealed a higher *MYC* copy in COLO 320DM cells growing as a tumor in the nude mouse than in cell culture. This observation suggests that elevated *MYC* expression provides a growth or survival advantage to COLO 320DM cells *in vivo*. Cytogenetic observations on mouse SEWA tumors (34), mouse Rous sarcomas (35), and rat sarcomas (36) are consistent with this inference. Since differentiation of several tumors has been reported to coincide with reduced oncogene expression (e.g., see refs. 37 and 38), we speculate that eliminating *MYC* may enable cells with reduced *MYC* expression to terminally differentiate and consequently to be removed from the dividing population that contributes to tumor formation. The capability to eliminate extrachromosomally amplified oncogenes provides an additional strategy for investigating the relationship between oncogene overexpression, cellular differentiation, and the relevant signal transduction pathways involved.

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1. Stark, G. R., Debatisse, M., Giulotto, E. & Wahl, G. M. (1989) *Cell* **57**, 901–908.
2. Brodeur, G. M., Seeger, R. C., Schwab, M., Varmus, H. E. & Bishop, J. M. (1984) *Science* **224**, 1121–1124.
3. Slamon, D. J., Clark, G. M., Wong, S. G., Levin, W. J., Ullrich, A. & McGuire, W. L. (1987) *Science* **235**, 177–182.
4. Slamon, D. J., Godolphin, W., James, L. A., Holt, J. A., Wong, S. U., Keith, D. E., Levin, W. J., Stuart, S. G., Udove, J., Ullrich, A. & Press, M. F. (1989) *Science* **244**, 707–712.
5. Berns, E. M., Klijn, J. G. M., van Putten, W. R. J., van Staveren, I. L., Portengen, H. & Foekens, J. A. (1992) *Cancer Res.* **529**, 1107–1113.
6. Varley, J. M., Walker, R. A., Casey, G. & Brammar, W. J. (1988) *Oncogene* **3**, 87–91.
7. Yoshida, M. C., Wada, M., Satoh, H., Yoshida, T., Sakamoto, H., Miyagawa, K., Yokota, J., Koda, J., Kakinuma, M., Sugimura, T. & Terada, M. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 4861–4864.
8. Watson, P. H., Pon, R. T. & Shiu, R. P. C. (1991) *Cancer Res.* **51**, 3996–4000.
9. Trent, J., Buick, R. N., Olson, S., Horns, R. C. & Schimke, R. T. (1984) *J. Clin. Oncol.* **2**, 8–15.
10. Benner, J. E., Wahl, G. M. & Von Hoff, D. D. (1991) *Anticancer Drugs* **2**, 11–25.
11. Snapka, R. M. & Varshavsky, A. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 7533–7537.
12. Von Hoff, D. D., Waddelow, T., Forseth, B., Davidson, K., Scott, J. & Wahl, G. (1991) *Cancer Res.* **51**, 6273–6279.
13. Collins, S. J., Gallo, R. C. & Gallagher, R. F. (1977) *Nature (London)* **270**, 347–349.
14. Von Hoff, D. D., Needham-VanDevanter, D. R., Yucel, J., Windle, B. E. & Wahl, G. M. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 4804–4808.
15. Von Hoff, D. D., Forseth, B. J., Clare, N., Hansen, K. L. & VanDevanter, D. R. (1990) *J. Clin. Invest.* **85**, 1887–1895.
16. Quinn, L. A., Moore, G. F., Morgan, R. T. & Woods, L. K. (1979) *Cancer Res.* **39**, 4914–4924.
17. VanDevanter, D. R., Piaskowski, V. D., Casper, J. T., Douglass, E. C. & Von Hoff, D. D. (1990) *J. Natl. Cancer Inst.* **82**, 1815–1821.
18. Trent, J., Meltzer, P., Rosenblum, M., Harsh, G., Kinzler, K., Mashal, R., Feinberg, A. & Vogelstein, B. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 470–473.
19. Von Hoff, D. D., Clark, G. M., Stogdill, B. J., Sarosdy, M. F., O'Brien, M. T., Casper, J. T., Mattox, D. E., Page, C. P., Cruz, A. B. & Sandbach, J. F. (1983) *Cancer Res.* **43**, 1926–1931.
20. Naylor, S. L., McGill, J. R. & Zabel, B. (1987) *Methods Enzymol.* **151**, 279–292.
21. Pinkel, D., Straume, T. & Gray, J. W. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 2934–2938.
22. MacPherson, I. & Montagnier, L. (1964) *Virology* **23**, 291–294.
23. Freedman, V. H. & Shin, S.-I. (1974) *Cell* **3**, 355–359.
24. Kaufman, R. J., Brown, P. C. & Schimke, R. T. (1981) *Mol. Cell. Biol.* **1**, 1084–1093.
25. Schimke, R. T., Brown, P. C., Kaufman, R. J., McGregor, M. & Slate, D. L. (1980) *Cold Spring Harbor Symp. Quant. Biol.* **45**, 785–798.
26. Levan, A. & Levan, G. (1978) *Heriditas* **88**, 81–92.
27. Wani, M. A., Strayer, J. M. & Snapka, R. M. (1990) *Anticancer Drugs* **1**, 67–75.
28. Wani, M. A. & Shapka, R. M. (1990) *Cancer Invest.* **8**, 587–593.
29. Brown, P. C., Tlsty, T. D. & Schimke, R. T. (1983) *Mol. Cell. Biol.* **3**, 1097–1107.
30. Windle, B., Draper, B. W., Yin, Y., O'Gorman, S. & Wahl, G. M. (1991) *Genes Dev.* **5**, 160–174.
31. Hahn, P., Kapp, L. N., Morgan, W. F. & Painter, R. B. (1988) *Cancer Res.* **48**, 4607–4611.
32. Becker, P., Scherthan, H. & Zankl, H. (1990) *Gene Chromosomes Cancer* **2**, 59–62.
33. Miller, B. M., Zitzelsberger, H. F., Weier, H., Ul, G. & Adler, I. D. (1991) *Mutagenesis* **6**, 297–302.
34. Levan, G., Mandahl, N., Bengtsson, B. O. & Levan, A. (1977) *Hereditas* **86**, 75–90.
35. Mark, J. (1970) *Hereditas* **65**, 59–82.
36. Levan, G. & Levan, A. (1975) *Hereditas* **79**, 161–198.
37. Boise, L. H., Grant, S. & Westin, E. H. (1992) *Cell Growth Differ.* **3**, 53–61.
38. Imaizumi, M. & Breitman, T. R. (1988) *Cancer Res.* **48**, 6733–6738.