Molecular Mechanisms of Drug Resistance Involving Ribonucleotide Reductase: Hydroxyurea Resistance in a Series of Clonally Related Mouse Cell Lines Selected in the Presence of Increasing Drug Concentrations

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ABSTRACT

Mammalian ribonucleotide reductase is a highly regulated, rate-limiting activity responsible for converting ribonucleoside diphosphates to the deoxyribonucleotide precursors of DNA. The enzyme consists of two nonidentical proteins often called M1 and M2, both of which are required for activity. Hydroxyurea is an antitumor agent which inhibits ribonucleotide reductase by interacting with the M2 component specifically at a unique tyrosyl free radical. To obtain further information about drug resistance mechanisms, we have used M1 and M2 complementary DNAs and monoclonal antibodies to investigate the properties of a series of clonally related drug-resistant mouse cell lines, selected by a step-wise procedure for increasing levels of resistance to the cytotoxic effects of hydroxyurea. Several interesting mechanisms have been identified. Each successive drug selection step leading to the isolation of highly resistant cells was accompanied by detectable changes in cellular resistance and ribonucleotide reductase activities. The changes that occurred at each step involved the M2 component. A very early event, occurring at the first step in the selection process, was the amplification of the M2 gene accompanied by an increase in M2 messenger RNA. Although cellular resistance and M2 protein levels increased significantly during drug selection, only a modest change in M2 gene copy number was observed after the initial selection step. Analysis of wild-type, moderately resistant, and highly resistant cells indicated that, in addition to M2 gene amplification, posttranscriptional modification also occurred during drug selection. This second mechanism was not due to alterations in protein M2 half-life, but involved an increase in translational efficiency. By increasing the rate of M2 synthesis, without altering degradation rates, resistant cells were able to accumulate high levels of this key regulatory protein. Cells selected for the ability to proliferate in concentrations of drug as high as 4 mM exhibited changes that involved M2, without detectable changes to M1. These results provide further evidence that M1 and M2 levels are controlled by different mechanisms in mammalian cells. Eventually, however, cells required an elevation in the M1 protein, as well as the M2 protein, to survive in a hydroxyurea concentration of 5 mM. These results illustrate the complexity of the drug-resistant phenotype and provide further information about the molecular processes that lead to the development of cells resistant to low, intermediate, and high concentrations of hydroxyureas.

INTRODUCTION

The antitumor agent, hydroxyurea, enters mammalian cells by a diffusion process (1), specifically inhibits DNA synthesis (2), and is cytotoxic to proliferating cells (2, 3). In general, this drug has been used clinically in the treatment of a wide range of solid tumors as well as acute and chronic leukemia (4, 5), and it has shown promise as a radiation potentiator (6), as a myelosuppressive agent in treating polycythemia vera (7), and in controlling the proliferation of psoriasis (8). The primary site of action for hydroxyurea is the highly regulated enzyme ribonucleotide reductase, which reduces ribonucleotides to provide the deoxyribonucleotides required for the synthesis of DNA (3, 9). This reaction is rate limiting for DNA synthesis, and therefore the enzyme plays an important role in the regulation of cell division (3, 9). In mammalian cells, this enzyme consists of two nonidentical subunits often called M1 and M2 (10, 11). Protein M1 is a dimer with a molecular weight of 170,000 and contains substrate and effector binding sites (11). Protein M2 is a dimer with a molecular weight of 88,000 and contains stoichiometric amounts of non-heme iron and a unique tyrosyl free radical essential for reductase activity (12). Hydroxyurea inhibits ribonucleotide reductase by interacting with the M2 subunit to inactivate the tyrosyl free radical (13), and it has been useful as a selective agent in cell culture for the isolation of drug-resistant cell lines (3). In an earlier study of hydroxyurea resistance, we described the cellular properties of a highly resistant mouse cell line, which was selected in the presence of increasing concentrations of drug by a stepwise procedure (14, 15). At various steps during the selection, cells were saved for further study. The molecular events that lead to the development of cells exhibiting drug-resistant characteristics are of much interest. To obtain further insight into these processes, we have used recently prepared cDNA5 clones encoding the M1 and M2 subunits (16) and monoclonal antibodies to investigate the molecular properties of these clonally related mouse cell lines, which exhibited low, intermediate, or high drug-resistant characteristics. Several interesting mechanisms important in the development of drug resistance have been identified.

MATERIALS AND METHODS

Cell Lines and Culture Conditions. Mouse L-cells were cultured at 37°C on plastic tissue culture plates (Lux Scientific) in α-minimal essential medium (Flow Laboratories) supplemented with antibiotics and 10% (v/v) fetal calf serum (Gibco). Colony-forming efficiencies were determined as previously described (17). Relative colony-forming efficiency was defined as the colony-forming efficiency in the presence of drug divided by that in the absence of drug. The Dα value is the drug concentration which reduces relative colony-forming efficiency to 10%. The method used to isolate a series of mouse cell lines with gradually increasing drug resistance properties has been described (14). Starting with the wild-type population, the following drug concentrations were used in the selection of hydroxyurea-resistant lines: 0.35 mM (H3PO4·3H2O); 1.3 mM (H3PO4·3H2O); 15.5 mM (H3PO4·3H2O); 2.0 mM (H3PO4·3H2O); 3.0 mM (H3PO4·3H2O); 4.0 mM (H3PO4·3H2O); 5.0 mM (H3PO4·3H2O). For the sake of clarity, the LHF cell line will be referred to as H1.5.5 in the present study. At each selection step between 1.3 mM and 5.0 mM hydroxyurea, cells were frozen in the

5 The abbreviations used are: cDNA, complementary DNA; DTG, dithiothreitol; EPR, electron paramagnetic resonance; SDS, sodium dodecyl sulfate; TBA, tetrabutylammonium; H3PO4·3H2O, sodium dihydrogen phosphate; NaCl, sodium chloride; Tris·Cl, pH 7.6, in 150 mM NaCl and 0.5% Tween 20; SSC, standard saline citrate.
presence of growth medium containing 5% dimethyl sulfoxide and stored at -70°C. Cells selected in 0.35 mM drug, were resolated from the wild-type population at a later date and also stored at -70°C. To carry out the present study, the cell lines were thawed, put into cell culture, and maintained in the absence of hydroxyurea as described above. The drug resistance properties of the various lines were stable and did not change during approximately 1 yr of continuous culture in the absence of a selective agent.

Preparation and Assay of Ribonucleotide Reductase. Enzyme preparations used for ribonucleotide reductase assays were obtained from wild-type cells and the various drug-selected cell lines (14, 15). Preparations containing 1 to 3 mg of protein/ml were used to measure CDP reductase activity by the method of Steeper and Stuart (18), using [³²P]CDP as substrate and snake venom to hydrolyze the nucleotides as previously described (14, 15, 17). The reaction mixture contained in a final volume of 150 µl: [³²P]CDP (0.05 µCi, 7.5 nmol); DTT (900 nmol); magnesium acetate (600 nmol); ATP (300 nmol); and a quantity of enzyme preparation. Reactions were initiated by the addition of enzyme and then carried out for 30 min at 37°C for CDP reductase and 1 h at 37°C in the presence of snake venom. Both reactions were terminated by boiling for 4 min. The heat-precipitated material was removed by centrifugation, and the deoxycytidine formed was separated from other labeled compounds on Dowex-1-borate (Bio-Rad) columns. Radioactivity was determined by liquid scintillation spectrometry.

EPR Spectroscopy. Cells in exponential growth were removed from the surface of culture plates, washed, transferred to EPR tubes (Wilmad Glass Co.), centrifuged, and stored in liquid nitrogen as previously described (16, 19). EPR first-derivative spectra were recorded at 77K on a Varian E-109 spectrometer equipped with an ER4040 XR microwave bridge and cold-finger Dewar with liquid nitrogen (20). Determinations of free radical concentrations in samples were performed by comparing the EPR signals of these samples with a control signal of packed M2 overproducing mouse mammary tumor TAJ3 cells, which were previously shown to have a tyrosine free radical concentration of 1.4 µM (16).

Southern and Northern Blot Analysis. Genomic DNA was prepared from logographically growing cells by phenol-chloroform extraction (21). For Southern analysis, 20 µg of genomic DNA were digested to completion with EcoRI or HindIII endonucleases (Boehringer Mannheim) followed by fractionation on 0.75% agarose gels and subsequently transferred to nitrocellulose membranes (Bio-Rad). Total cellular RNA was extracted from logographically growing cells by the guanidinium thiocyanate/cesium chloride method (22). Twenty-five µg of total cellular RNA were electrophoresed through 1% formaldehyde-agarose gels followed by electroblotting onto Zeta-probe nylon membranes (Bio-Rad). Total cellular RNA was extracted from logographically growing cells by the guanidinium thiocyanate/cesium chloride method (22). Twenty-five µg of total cellular RNA were electrophoresed through 1% formaldehyde-agarose gels followed by electroblotting onto Zeta-probe nylon membranes (Bio-Rad). Total cellular RNA was extracted from logographically growing cells by the guanidinium thiocyanate/cesium chloride method (22). Twenty-five µg of total cellular RNA were electrophoresed through 1% formaldehyde-agarose gels followed by electroblotting onto Zeta-probe nylon membranes (Bio-Rad). Total cellular RNA was extracted from logographically growing cells by the guanidinium thiocyanate/cesium chloride method (22). Twenty-five µg of total cellular RNA were electrophoresed through 1% formaldehyde-agarose gels followed by electroblotting onto Zeta-probe nylon membranes (Bio-Rad). Total cellular RNA was extracted from logographically growing cells by the guanidinium thiocyanate/cesium chloride method (22). Twenty-five µg of total cellular RNA were electrophoresed through 1% formaldehyde-agarose gels followed by electroblotting onto Zeta-probe nylon membranes (Bio-Rad). Total cellular RNA was extracted from logographically growing cells by the guanidinium thiocyanate/cesium chloride method (22). Twenty-five µg of total cellular RNA were electrophoresed through 1% formaldehyde-agarose gels followed by electroblotting onto Zeta-probe nylon membranes (Bio-Rad). Total cellular RNA was extracted from logographically growing cells by the guanidinium thiocyanate/cesium chloride method (22). Twenty-five µg of total cellular RNA were electrophoresed through 1% formaldehyde-agarose gels followed by electroblotting onto Zeta-probe nylon membranes (Bio-Rad). Total cellular RNA was extracted from logographically growing cells by the guanidinium thiocyanate/cesium chloride method (22). Twenty-five µg of total cellular RNA were electrophoresed through 1% formaldehyde-agarose gels followed by electroblotting onto Zeta-probe nylon membranes (Bio-Rad). Total cellular RNA was extracted from logographically growing cells by the guanidinium thiocyanate/cesium chloride method (22). Twenty-five µg of total cellular RNA were electrophoresed through 1% formaldehyde-agarose gels followed by electroblotting onto Zeta-probe nylon membranes (Bio-Rad). Total cellular RNA was extracted from logographically growing cells by the guanidinium thiocyanate/cesium chloride method (22). Twenty-five µg of total cellular RNA were electrophoresed through 1% formaldehyde-agarose gels followed by electroblotting onto Zeta-probe nylon membranes (Bio-Rad). Total cellular RNA was extracted from logographically growing cells by the guanidinium thiocyanate/cesium chloride method (22). Twenty-five µg of total cellular RNA were electrophoresed through 1% formaldehyde-agarose gels followed by electroblotting onto Zeta-probe nylon membranes (Bio-Rad). Total cellular RNA was extracted from logographically growing cells by the guanidinium thiocyanate/cesium chloride method (22). Twenty-five µg of total cellular RNA were electrophoresed through 1% formaldehyde-agarose gels followed by electroblotting onto Zeta-probe nylon membranes (Bio-Rad). Total cellular RNA was extracted from logographically growing cells by the guanidinium thiocyanate/cesium chloride method (22). Twenty-five µg of total cellular RNA were electrophoresed through 1% formaldehyde-agarose gels followed by electroblotting onto Zeta-probe nylon membranes (Bio-Rad). Total cellular RNA was extracted from logographically growing cells by the guanidinium thiocyanate/cesium chloride method (22). Twenty-five µg of total cellular RNA were electrophoresed through 1% formaldehyde-agarose gels followed by electroblotting onto Zeta-probe nylon membranes (Bio-Rad).
sequentially selected in the presence of increasing concentrations of hydroxyurea for the ability to proliferate in normally cytotoxic drug concentrations, as described in "Materials and Methods." The colony-forming abilities of the parental wild-type population and the various drug-selected cell lines in the presence of drug-supplemented medium are shown in Fig. 1. All drug-selected cell lines exhibited a reduced sensitivity to the cytotoxic effects of hydroxyurea when compared to parental wild-type cells. For example, when the \( D_{10} \) values for hydroxyurea are considered (Table 1), cells selected in the presence of the lowest drug concentration (0.35 mM hydroxyurea) exhibited approximately an 8-fold elevation in drug resistance, whereas cells selected at the highest concentration (5 mM hydroxyurea) were about 35-fold more drug resistant than the wild-type population. Furthermore, the drug resistance properties of the cell lines significantly increased at each of the drug selection steps tested (0.35 mM, 1.5 mM, 2.0 mM, 3.0 mM, 4.0 mM, and 5.0 mM hydroxyurea). In keeping with the resistance characteristics, each drug-selected line also exhibited an elevation in ribonucleotide reductase activity when compared to the wild-type cell line (Table 1). There was a sequential increase in enzyme activity with each selection step, with the least resistant line showing a modest increase in activity, and the highest resistant line exhibiting the greatest increase in enzyme activity of approximately 24-fold.

Analysis of \( M_1 \) and \( M_2 \) DNA from Wild-Type and Mutant Cell Lines. To compare the relative number of \( M_1 \) and \( M_2 \) gene copies in wild-type and drug-resistant cells, DNA isolated from the various lines was digested to completion with EcoRI or HindIII endonucleases, and Southern blots of the digested mixtures were hybridized with \( ^{32} \)P-labeled \( M_1 \) or \( M_2 \) cDNA probes (Fig. 2, A and B). There were no indications of \( M_1 \) gene amplification in any of the drug-resistant cell lines, since the intensity of the restriction bands was about the same with DNA obtained from wild-type and drug-resistant populations (Fig. 2A). The similarity of the banding patterns also suggested that there were no obvious \( M_1 \) gene rearrangements in any of the drug-resistant cell lines. In contrast to the above observations, the \( M_2 \) gene was clearly amplified in all the drug-resistant populations (Fig. 2, B and C). None of the cell lines appeared to contain gross \( M_2 \) gene rearrangements. As observed in previous studies of drug-resistant cell lines (19), only selected bands were amplified (Fig. 2B), and this may be due to the presence of \( M_2 \) pseudogenes in mammalian cells (33). Densitometry of the most intense bands of the \( M_2 \) cDNA-probed autoradiograms gave estimates of 1.5, 2.9, 3.0, 3.4, 3.8, and 3.9 more hybridization with DNA from \( H^* \)-0.35, \( H^* \)-1.5, \( H^* \)-2.0, \( H^* \)-3.0, \( H^* \)-4.0, and \( H^* \)-5.0 cells, respectively, when compared to the wild-type condition. It is interesting to note (Fig. 2, B and C) that there was relatively little change (approximately 35%) in hybridization intensity with DNA isolated from cells selected between 1.5 mM and 5.0 mM hydroxyurea (between \( H^* \)-1.5 and \( H^* \)-5.0 cells). This is in contrast to the significant increases in the levels of cellular resistance and ribonucleotide reductase activities that occur during selection of these drug-resistant lines (Table 1).

Analysis of \( M_1 \) and \( M_2 \) Message Levels in Wild-Type and Mutant Cell Lines. Northern blot analysis using \( M_1 \) cDNA is presented in Fig. 3A. These results are consistent with other investigations of mammalian cell lines which showed the presence of a single \( M_1 \) mRNA species of about 3.1 kilobases (16, 19). Although no major changes in \( M_1 \) mRNA concentrations were detected in most of the drug-resistant cell lines, an increase of about 1.8 fold, as determined by densitometry analysis, was routinely observed when RNA from wild-type and \( H^* \)-5.0 cells was compared. Interestingly, \( M_2 \) cDNA-probed Northern blots, presented in Fig. 3B, showed significant elevations in the \( M_2 \) mRNA levels in all the resistant cell lines. A 2.1-kilobase \( M_2 \) mRNA species was clearly observed in both wild-type and mutant cell lines; upon longer exposure a 1.6-kilobase \( M_2 \)

![Graph](image_url)
mRNA species also became evident in all the cell lines. Thelander and Berg (16) and Wright et al. (19) have previously described the presence of two M2 mRNA species of 2.1 and 1.6 kilobases in mouse cell lines, and they have suggested that these species may be generated through the use of different polyadenylation sites.

Quantitation of mRNA levels was carried out by densitometry of dot blots (25, 34) of total cellular RNA probed with either M1 or M2 cDNA (Table 1). In agreement with Northern blot analysis (Fig. 3A) only the H^2.5 cell line showed a significant increase in M1 message of about 20-fold. Also, in agreement with Northern blot studies (Fig. 3B) large elevations in M2 mRNA levels were observed in all drug-resistant lines when compared to the wild-type population. The least resistant, H^2.35 line, showed an 11-fold increase in M2 mRNA concentration, and interestingly, the rest of the drug-resistant lines (H^3.0, H^3.5, H^4.0, and H^5.0) had approximately the same elevation in the M2 message of about 23-fold (Table 1).

EPR Spectroscopy and Immunoblot Analysis. The increase in M2 mRNA levels in drug-resistant lines suggested that these cells contained elevations in the M2 component. Since this protein has a tyrosine free radical necessary for enzyme activity, it is possible to determine expression of M2 protein in whole cells by measuring the free radical signal characteristic of a functional M2 component (16, 19, 25). An EPR analysis was
performed on three representative cell lines: the wild-type line; the moderately resistant Hr-1.5 line; and the most resistant line, Hr-5.0. The concentration of free radical during exponential growth was 0.14 μM, 1.2 μM, and 7.0 μM for wild-type, Hr-1.5, and Hr-5.0 cells, respectively. Although Hr-1.5 and Hr-5.0 lines contained about the same level of M2 mRNA (Fig. 3B; Table 1), there was a significant difference between these two cell lines in tyrosyl free radical content. Recent purification of the M2 protein and preparation of specific M2 monoclonal antibodies (12, 25) have provided the ability to directly measure the levels of M2 protein by Western blot analysis. Cell extracts prepared from wild-type, Hr-1.5, and Hr-5.0 cells were subjected to SDS-polyacrylamide gel electrophoresis and blotted onto nitrocellulose, and M2 protein was detected using anti-M2 monoclonal antibody (Fig. 4A). The results indicated that a M, 44,000 band corresponding to M2 protein was markedly overproduced in both drug-resistant cell lines. The increases observed in the Western blots correlated with the 8.6-fold and 50-fold elevations in tyrosyl free radical content determined by EPR spectroscopy (Table 1). These results are also consistent with previous observations that there is normally no pool of radical-free M2 protein present in mammalian cells (20, 25).

The availability of monoclonal antibodies specific for protein M1 (24) makes it possible to compare the levels of the M1 protein in wild-type, Hr-1.5, and Hr-5.0 cells by Western blot analysis. The results of these experiments are shown in Fig. 4B. The anti-M1 mouse monoclonal antibody detected a high-molecular-weight band of about 90,000, corresponding to protein M1. The level of M1 in the Hr-1.5 cells did not change from that of the wild-type cells, whereas the highly resistant Hr-5.0 cell line showed an approximate 2-fold increase in M1 protein over the wild-type line. 

Posttranscriptional Modulation of Protein M2. The difference in protein M2 pools between Hr-1.5 and Hr-5.0 cells, given the similar levels of M2 mRNA, suggests that changes in M2 protein occur by a posttranscriptional mechanism, such as an increase in half-life or through an increase in protein M2 biosynthetic rate in the highly resistant line. Protein turnover was measured in [35S]methionine pulse-chase experiments as described in “Materials and Methods.” These studies indicated that the M2 protein half-life was 6.3 h in both Hr-1.5 and Hr-5.0 cells and was unchanged from the value determined for the wild-type M2 protein (Fig. 5A). Also, as expected, no differences in the M1 protein half-life of 12.5 h were found (Fig. 5B). The rates of M2 protein biosynthesis in the Hr-1.5 and Hr-5.0 cells were estimated following a 20-min pulse labeling with [35S]methionine and immunoprecipitation with JB4 anti-M2 monoclonal antibody as described in “Materials and Methods.” The results of such an immunoprecipitation are shown in Fig. 6. The M2 protein band with an apparent molecular weight of 44,000 is clearly visible. Densitometric quantitation of the bands indicated that there was approximately a 2-fold increase in the metabolically labeled M2 immunoprecipitate in the Hr-5.0 cell line compared to the Hr-1.5 line. This increase in protein M2 biosynthetic rate would contribute to the elevation in cellular M2 content observed in the Hr-5.0 cell line (Fig. 4).

**DISCUSSION**

Ribonucleotide reductase is responsible for providing a continuous and balanced supply of deoxyribonucleotide precursor essential for DNA synthesis. Enzyme inhibitors such as the chemotherapeutic agent, hydroxyurea, inhibit DNA synthesis through ribonucleotide reductase, by altering deoxyribonucleotide concentrations, the substrates for DNA polymerase (35). A mechanism for achieving resistance to hydroxyurea and related drugs is through elevation of ribonucleotide reductase activity. This ensures that cells with high levels of enzyme activity will still have enough active enzyme, when grown in the presence of normally cytotoxic concentrations of drug, to provide the required deoxyribonucleotides for the synthesis of DNA.

Although ribonucleotide reductase contains the two non-identical subunits M1 and M2, the precise site of action of hydroxyurea is at the M2 subunit, where the drug specifically destroys a tyrosine free radical needed for enzyme activity. The M1 subunit appears to be nearly constant throughout the cell cycle of proliferating cells, whereas de novo synthesis of M2 correlates with S phase, suggesting that the reduction of ribonucleotides is directly dependent upon the concentration of M2.
protein (20, 36). These observations are in keeping with our findings that hydroxyurea-resistant cell lines with elevated levels of enzyme activity frequently contain increased levels of M2 (14, 16, 19). This point is strongly supported in the present study. Each successive drug selection step leading to the isolation of highly resistant cells was accompanied by stable increases in cellular resistance and ribonucleotide reductase activities. Furthermore, enzyme alterations at each step involved the M2 protein. Observations that changes in M2 occur independently of M1 in drug-resistant cells are consistent with studies showing that M1 and M2 levels are controlled by different mechanisms in mammalian cells (19, 36) and are in marked contrast to the situation in Escherichia coli, where the two equivalent genes are located in one operon and their expression is coordinately regulated (37).

Several different molecular mechanisms were involved in the development of the hydroxyurea-resistant phenotype. A very early event, occurring at the first step in the selection process, was the amplification of the M2 gene which was accompanied by an elevation in M2 mRNA. There appeared to be a further increase in gene copy number after the first selection step (0.35 mM hydroxyurea), but only a modest change in M2 gene copies or mRNA levels was detected during the remaining selection steps (1.5 mM, 2.0 mM, 3.0 mM, 4.0 mM, and 5.0 mM drug). These observations contrast with the finding that cellular resistance increased significantly, especially during the latter stages of drug selection. Also, highly drug-resistant cells do not appear to possess an enzyme activity less sensitive to hydroxyurea (14). EPR studies and Western blot analysis revealed that M2 pool sizes were markedly different among wild-type, moderately resistant, and highly resistant cells, indicating that, in addition to gene amplification, posttranscriptional modification occurred during selection of hydroxyurea-resistant cells. This additional mechanism was not due to changes in protein M2 half-life, but instead involved an increase in the M2 protein biosynthetic rate. Total cellular protein synthesis, measured by incorporation of [35S]methionine into proteins, was essentially unchanged in the H8-1.5 and H8-5.0 cell lines (6.0 x 10^6 cpm/μg for H8-1.5 and 6.1 x 10^6 cpm/μg for H8-5.0 cells), which attests to the selectivity of increased M2 biosynthesis in H8-5.0 cells. Therefore, by increasing the rate of protein M2 biosynthesis, without changing the rate of degradation, cells can accumulate very high levels of this key protein. Interestingly, this mechanism of increasing protein biosynthetic rates in the absence of mRNA elevations has also been observed in other cell lines that overproduce highly regulated proteins, such as ornithine decarboxylase (38) or dihydrofolate reductase (39).

Cells selected for the ability to proliferate in the presence of hydroxyurea concentrations as high as 4 mM exhibit changes that only involved the M2 subunit without detectable alterations to M1. However, both M1 and M2 are required for enzyme activity. It appears that the M2 subunit is limiting for activity in wild-type mouse L-cells, but as the level of M2 increases during selection of drug-resistant variants, the M1 protein eventually becomes the limiting component for activity. This view is consistent with the observation that cells require an increase in the M1 protein, as well as M2, in order to survive concentrations of drug as high as 5.0 mM. Thus, an approximately 2-fold increase in M1 mRNA and protein was observed in the H8-5.0 cell line, and this was probably important in achieving the very large elevation in ribonucleotide reductase activity observed with these cells.

In conclusion, we have observed that an early event in the establishment of a hydroxyurea-resistant phenotype involves amplification of the M2 gene of ribonucleotide reductase. As cells become increasingly more resistant to this drug, other processes controlling the expression of the two subunits of ribonucleotide reductase are also altered (see Table 1). Furthermore, this study suggests that multiple mechanisms involving the regulation of the two proteins of ribonucleotide reductase are important determinants of chemotherapeutic sensitivity.

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DRUG RESISTANCE MECHANISMS INVOLVING RIBONUCLEOTIDE REDUCTASE


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