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HYPERPRODUCTION OF A SPECIFIC PROTEIN IN CELLS RESISTANT TO COLCHICINE AND ADRIABLASTIN

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UDC 616-008.939.6-008.61-02:[615.277.2+615.332

KEY WORDS: colchicine; adriablastin; gene amplification; protein synthesis; permeability of the plasma membrane.

Resistance of mammalian somatic cells to certain cytotoxic agents (methotrexate, N-phosphoacetyl-L-aspartate, etc.) arises as a result of amplification of the genes of proteins whose hyperproduction is responsible for the development of drug resistance [7, 9, 11, 12]. The writers showed previously that resistance of Djungarian hamster and mouse cells to colchicine is connected with gene amplification, leading to a decrease in permeability of the plasma membrane for colchicine and certain other substances [2-4, 6]. It has also been found that approximately the same changes in genotype and plasma membrane permeability take place in cells selected for resistance to adriamycin as in cells isolated in the presence of toxic concentrations of colchicine [4].

In the investigation described below a protein hyperproduced in colchicine-resistant and adriablastin-resistant cells, containing amplified genes, was identified by two-dimensional electrophoresis.

EXPERIMENTAL METHOD

Djungarian hamster cells of line DM-15 [1], colchicine-resistant cells of sublines $DM^{5/1}$ and $DM^{5/5}$ [2, 3], and adriablastin-resistant cells of subline $DM^{adb-0.8}$ [4], obtaining from them, and also mouse cells of lines L and L-53 (a subline of L cells resistant to colchicine [5]), were used.

The conditions of culture of the cells and the degree of their drug resistance were described previously [2-4].

Proteins from sensitive and resistant cells were analyzed by two-dimensional gel-electrophoresis by O'Farrell's method [10] with certain modifications. Cells growing on dishes 60

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 0007-4888/83/9609-1304$07.50

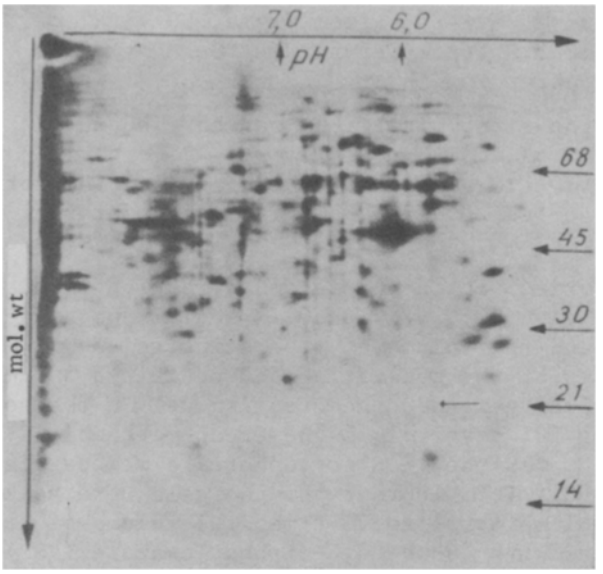

Fig. 1. Electrophoresis of proteins from line L-53 mouse cells, obtained by two-dimensional electrophoresis after labeling with [^{35}S]methionine. Abscissa, isoelectric point; ordinate, molecular weight in kilodaltons. Protein with molecular weight of about 22 kilodaltons and isoelectric point 5.7 is shown.

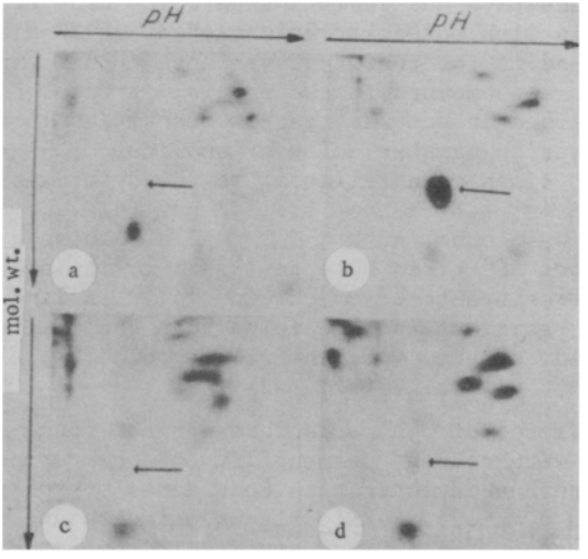

Fig. 2. Fragments of gel after electrophoresis of proteins. a) From Djungarian hamster cells of line DM-15, sensitive to colchicine; b) from Djungarian hamster cells of line DM$^{5/1}$, resistant to colchicine; c) from mouse cells of line L, sensitive to colchicine; d) from mouse cells of line L-53, resistant to colchicine. Arrow indicates position of protein with molecular weight about 22 kilodaltons and isoelectric point 5.7.

mm in diameter were labeled for 3 h with 100 μCi/ml of ^{35}S-methionine (Amersham Corporation, England; specific radioactivity 500 Ci/mmole) in 1 ml of Hanks' solution containing 0.025 M HEPES and 10% of dialyzed embryonic calf serum (from Flow Laboratories, England). The cells were washed with Hanks' solution, removed with a rubber spatula, and dissolved in 0.024 ml of a solution containing 3% sodium dodecylsulfate and 10% β-mercaptoethanol (from Serva, West Germany). To each sample was then added 0.025 ml of a solution containing 500 μg/ml DNase I and 500 μg/ml RNase A (from Serva, West Germany), 500 mM Tris-HCl, pH 7.0, and 50 mM $MgCl_2$. The samples were quickly frozen (−70°C) and lyophilized. The lyophilized samples were dis-

solved in 0.25 ml of buffer: 9.95 M urea (from Sigma, USA), 4% of NP-40, 100 mM dithiothreitol, and 2% ampholines with pH 5.0-8.0 (from Serva, West Germany), and the radioactivity of the acid-insoluble fraction was determined.

Isoelectric focusing (the first direction) was carried out in 4% acrylamide (ratio acrylamide:bisacrylamide 28.38:1.62), containing 0.4% of ampholines with pH 2.0-11.0 and 1.6% of ampholines with pH 5.0-8.0, in glass tubes 5 mm in diameter and 130 mm long (volume of gel about 2.5 ml). To each gel 0.012-0.020 ml of test material containing about 10^6 cpm was applied.

Electrophoresis was carried out in the second direction in plates of 12% acrylamide (acrylamide:bisacrylamide ratio 30:0.4) with 0.1% sodium dodecylsulfate. The volume of concentrating gel was 5 ml and the volume of separating gel 29 ml, and the thickness of the spacers was 2 mm. The buffer of the separating gel contained 500 mM Tris-HCl, pH 8.9. For contact between the cylindrical gel and concentrating gel in the second direction, 0.75% agarose in buffer containing 10% glycerol, 5% β-mercaptoethanol, 2.3% sodium dodecylsulfate, and 0.0625 M Tris-HCl, pH 6.8, was used. Electrophoresis was carried out at a voltage of 25 V to the plates for 15 h at 4°C. For fluorography the gels were soaked for 1 h in two changes of dimethylsulfoxide in a volume equal to 20 times the volume of the gel. During the next 3 h the gels were impregnated with 16% of 2,5-diphenyloxazole (PPO) in dimethylsulfoxide in a volume equal to 4 times the volume of the gel, and washed in several changes of water in the course of 1 h. The gels were dried in vacuo at 75°C and exposed on ORWO HS II x-ray film at −70°C.

EXPERIMENTAL RESULTS

The technique of two-dimensional electrophoresis used revealed 650-700 proteins synthesized in Djungarian hamster and mouse cells (Fig. 1). The difference between the electrophoresis patterns of cells sensitive and resistant to colchicine and adriablastin was that increased synthesis of a protein with molecular weight of about 22 kilodaltons and with an isoelectric point of 5.7 was found in the resistant variants in all experiments (Figs. 1 and 2). This protein was present in trace amounts in the initial parental Djungarian hamster cells of line DM-15 and it was synthesized in somewhat larger quantities in wild-type line L mouse cells (Fig. 2a, c). In Djungarian hamster cells of sublines $DM^{5/1}$ and $DM^{5/5}$, resistant to colchicine, which are 750-800 times more resistant to the cytotoxic action of the cytostatic, a sharp increase was observed in production of protein p22 (Fig. 2b). Considerable hyperproduction of this protein also was found in $DM^{adb-o.s}$ cells. These cells, selected in the presence of adriablastin are about 270 times more resistant to the selective agent than the wild-type cells and possess cross-resistance to colchicine [4]. In line L-53 mouse cells, which are about 150-200 times more resistant to colchicine than the initial L cells, synthesis of protein p22 was also increased compared with the wild-type cells, although by a lesser degree than in $DM^{5/1}$, $DM^{5/5}$, and $DM^{adb-o.s}$ cells (Fig. 2d).

Consequently, in all sublines of Djungarian hamster and mouse cells with resistance to various cytotoxic agents and with reduced permeability of their plasma membrane for these substances, studied in the present investigation considerable hyperproduction of a specific protein with molecular weight of about 22 kilodaltons and with an isoelectric point of 5.7 was found. Since drug resistance of all lines studied is connected with gene amplification [2-4, 6], it can be tentatively suggested that it is this phenomenon which lies at the basis of hyperproduction of protein p22 and of a change in permeability of the plasma membrane.

Amplification of the genes of protein p22 is a fairly common mechanism of protection against the cytotoxic action of various agents differing in their chemical structure and the mechanism of their toxic action. For instance gene amplification and similar changes in permeability of the plasma membrane and hyperproduction of protein p22 were found by the writers both in Djungarian hamster and mouse cells isolated in the presence of toxic concentrations of colchicine and in Djungarian hamster cells selected in the presence of adriablastin. Similar genotypic and phenotypic changes were found previously in vincristine-resistant Chinese hamster, mouse, and human cells [8].

In this connection a further study of protein p22 and, in particular, the elucidation of its function, are of great interest.

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