GENETICS

Effect on Different Mutant p53 on Cell Sensitivity to Cytostatics

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Expression of human p53 carrying missense mutations at codons 175 (His175), 194 (His194), 248 (Trp248), and 273 (His273) has different effects on sensitivity of K562, 10(1), 10(3), and Rat1 cells to the antitumor drugs methotrexate, etoposide, vinblastine. These effects of mutant p53 depend on both particular amino acid substitution and cell context (histogenetic type, status of the second allele, *etc.*)

Key Words: *p53; chemotherapy; methotrexate; vinblastine; etoposide*

Mutations of p53 tumor suppressor gene are present in 40-70% human neoplasms of more than 50 types [4,9], missense mutations (amino acid residue substitutions) being most common type of aberrations. These mutations primarily occur at codones 248, 273, and 175 [4,9]. Functional abnormalities in p53 can result in inactivation of some cell cycle check points, inhibition of p53-induced apoptosis [5,8], and, therefore, modulation of cell sensitivity to cytostatics (CS). However, the attempts of using p53 mutations as a prognostic test in chemotherapy yielded contradictory results. In the majority of studies p53 mutations were assessed using histochemical methods [1,11,13], which provide no possibility of identifying the type of mutations. However, the effect of different p53 mutations on cell sensitivity to different cytostatics and the role of cell context in this process (cell type and the state of the second p53 allele) remain unclear.

We studied the effect of expression of different mutant p53 on the sensitivity of various cell strains with different endogenous p53 status to 3 cytostatics: methotrexate (MT), vinblastine, and etoposide.

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MATERIALS AND METHODS

Experiments were carried out on K562 human erythroleukemic cells producing no p53 [10], mouse p53-negative embryonic fibroblasts 10(1) and 10(3) [3], immortalized rat fibroblasts Rat1 (express wild-type p53) and these cell sublines [6,7] transduced with different mutant p53 (His273, His175, and His194) and empty retroviral vector pPS/*neo*. The cells were cultured in DMEM or RPMI-1640 (for K562 cells) supplemented with 10% fetal bovine serum (humidified atmosphere with 5% CO_2).

The sensitivity of Rat1, 10(1), and 10(3) cell sublines carrying different p53 cDNA or empty vector pPS/neo was assessed by their ability to form clones in media containing different CS. To this end, the cells were seeded to 60-mm Petri dishes (Nunc, 200 cells per dish, 2 dishes for each series) and after 9-12 days colonies (>50 cells) were counted. The sensitivity of K562 cells was evaluated in suspension cultures by growth curves in the presence of varying concentrations of CS. To this end, 2×10^4 cells in 1 ml medium were seeded to 12-well plates (Nunc) and after 1, 3, 5, and 7 days the number of live cells was determined by the trypan blue exclusion test. The effect of CS on K562 cells was evaluated by the inhibition/stimulation coefficient: $(N_T\text{-}N_O)/N_O\times100\%$,

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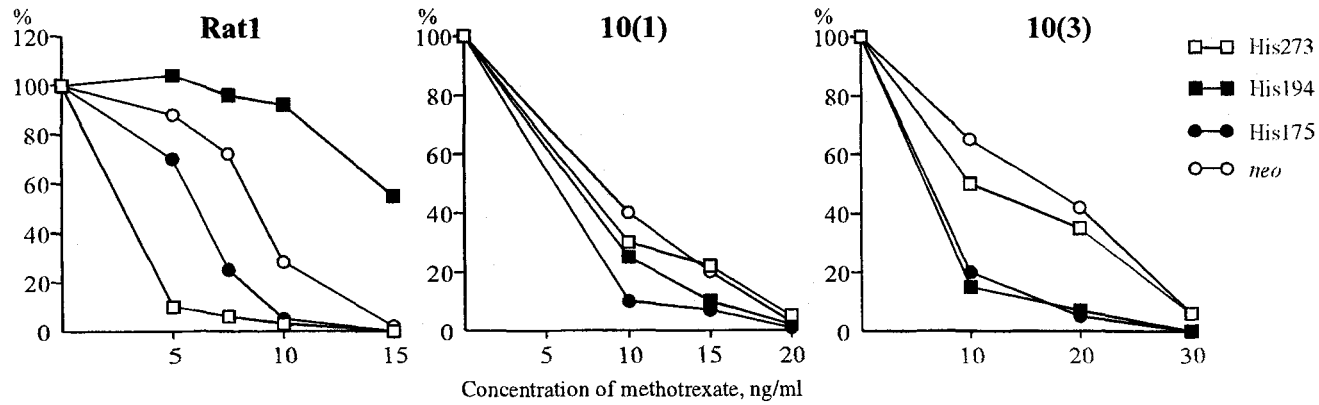

Fig. 1. Clone formation by Rat1, 10(1), and 10(3) cells expressing mutant p53 or empty retroviral vector *neo* in the presence of different concentrations of methotrexate. For each dose of methotrexate the relative number of colonies (ordinate) was calculated as the ratio of colonies formed in the presence to that in the absence of the drug.

where N_O and N_T are the number of seeded and survived (5 days) cells.

RESULTS

Expression of different p53 mutants had unequal effects on cell sensitivity to CS. For instance, colony-forming capacity of Rat1 cells carrying p53 with point mutations at codones 175 and 273, the most common p53 mutations in human neoplasms [4,9], in the presence of varying concentrations of MT was considerably decreased in comparison with that of control Rat/*neo* cells carrying empty vector pPS/*neo*. In contrast, expression of p53 with missense mutations at codone 194 reduced cell sensitivity to MT (Fig. 1).

In other cellular context, expression of exogenous p53-His175 and p53-His194 in p53-null mouse fibroblasts 10(1) and 10(3) produced the same effect: their clone-forming capacity in the presence of MT considerably decreased in comparison with control cells containing empty vector and expressing only the selection *neo* gene (Fig. 1). The effects of mutant p53-His273 in 10(1), 10(3), and Rat1 cells differed considerably: it increased sensitivity to CS in Rat1 cells and had no effect on 10(1) and 10(3) cells (Fig. 1).

Different effects of mutant p53 were observed also in K562 erythroleukemic cell sublines. In particular, expression of mutant p53-His175 that increased sensitivity to MT in fibroblasts (Fig. 1), had an opposite effect in erythroleukemia cells (Figs. 2 and 3). The expression of p53-His175 slightly increased sensitivity of K562 cells to vinblastine (Fig. 2), but did not modulate their resistance to etoposide (Figs. 2 and 3). Effects of other mutant p53 were minor, but K562 cell sensitivity to etoposide, vinblastine, and MT tended to increase (Figs. 2 and 3).

Thus, expression of p53 with missense mutations at different codones has opposite effects on cell sensitivity to antitumor drugs. Different effects were produced by various mutants in the same cell strain and by a certain mutant in different cells. In Rat1 cells containing both normal and mutant p53 alleles, the effect of p53 mutants on cell sensitivity to MT can differ due to their unequal ability to suppress function

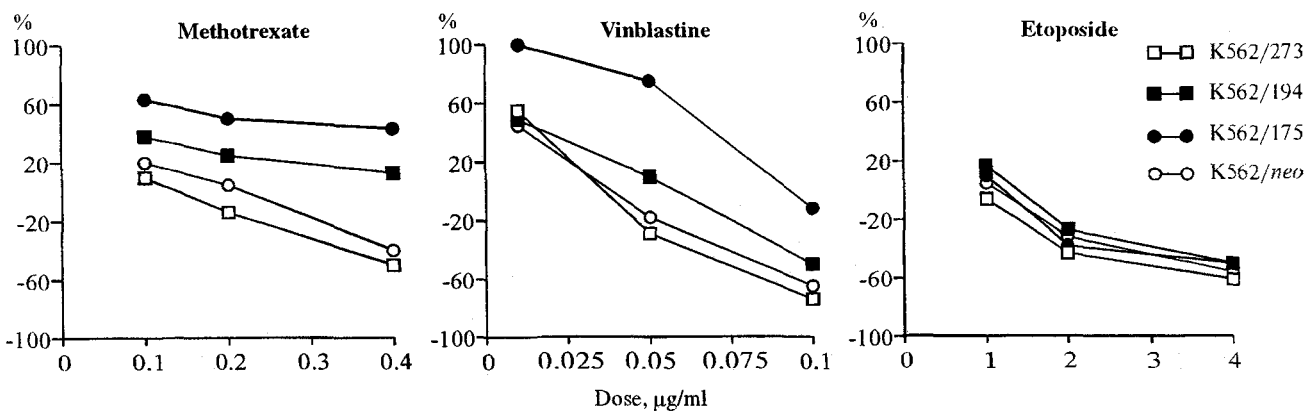

Fig. 2. Sensitivity of K562 cell sublines expressing mutant p53 or empty retroviral vector *neo* to methotrexate, vinblastine, and etoposide. Ordinate: % of inhibition.

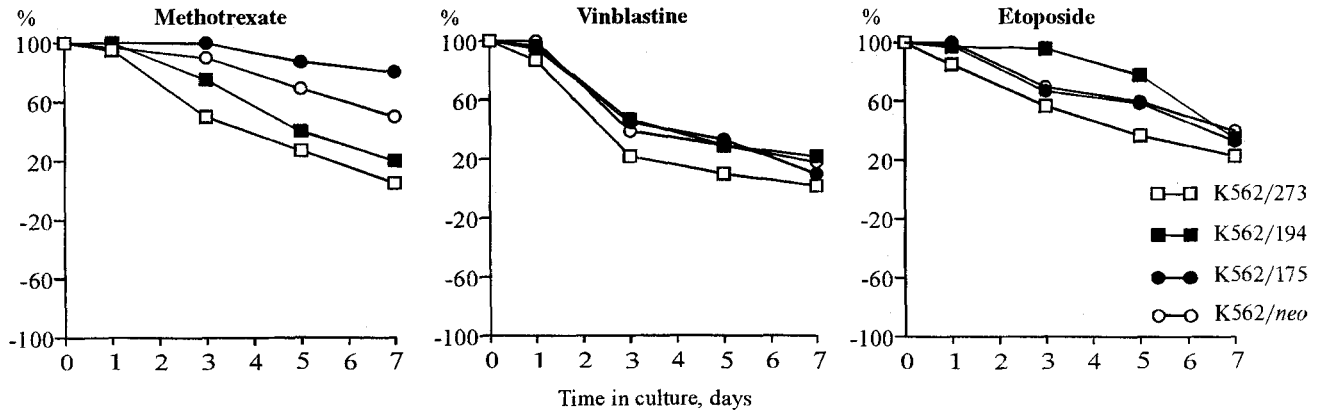

Fig. 3. Dynamics of cell viability in K562 cells expressing mutant p53 or empty retroviral vector *neo* in the presence of methotrexate (0.3 μg/ml), vinblastine (0.1 μg/ml), and etoposide (2 μg/ml). Number of seeded cells was taken as 100%. Ordinate: relative number of cells, %.

of wild-type p53 via a dominant-negative mechanism. It was shown that suppression of wild-type p53-induced transactivation by exogenous p53-His175 and p53-His273 considerably varied in different cell strains [2]. However, in our experiments mutant p53 modulated drug sensitivity in cells expressing no endogenous p53: some of them increased, while others decreased cells sensitivity to the same agent. These findings suggest that amino acid substitutions at different codones determine new properties of p53, which are responsible for different effects of these mutants. It should be noted that the effect of p53 mutants on the resistance to antitumor drugs, in particular MT, in p53-null fibroblasts considerably differed from that in p53-null erythroleukemia cells. Hence, manifestation of new properties of mutant p53 as well as their dominant-negative character largely depended on cellular context.

Thus, expression of p53 mutants has different effects on cell sensitivity to various antitumor drugs. These changes depend not only on the type of mutation but also on cellular context. This should be taken into account when using p53 mutations as a prognostic factor in predicting the outcome of antitumor therapy.

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