Mutant p53-dependent growth suppression distinguishes PRIMA-1 from known anticancer drugs: a statistical analysis of information in the National Cancer Institute database

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Introduction

The tumor suppressor p53 is a crucial mediator of cell cycle arrest and apoptosis in response to various forms of cellular stress, including DNA damage, oncogene activation, and hypoxia (2–5). At least 50% of human tumors harbor mutations in p53 (6). Most anticancer drugs in current clinical use except paclitaxel target primarily tumor cells that carry wild-type p53 and trigger apoptosis, and have mutant p53-dependent anti-tumor activity in vivo (1). We showed that PRIMA-1 can restore wild type conformation and specific DNA binding of mutant p53 proteins and has anti-tumor activity in vivo (1). Here we report the statistical analysis of the effect of PRIMA-1 on a panel of human tumor cell lines using information available in a database at the Developmental Therapeutics Program of the National Cancer Institute (NCI). We extracted growth inhibition profiles for PRIMA-1 and 44 known anticancer agents, p53 status of cell lines, population doubling time, and level of p53 protein expression from the NCI database. The data were analyzed by linear regression, Wilcoxon matched pairs test, and cluster analysis. In a subset of human cell lines derived from colon, ovarian, renal, and non-small cell lung cancer and melanoma, the level of mutant p53 expression correlated with cell population doubling time, \( r = -0.53 \), \( P < 0.018 \). The GI\(_{50}\) values for PRIMA-1 correlated with levels of mutant p53, \( r = -0.75 \), \( P = 0.0002 \). PRIMA-1 showed a statistically significant preference at \( P = 0.04 \) for growth inhibition of tumor cell lines expressing mutant p53 as compared with lines expressing wild-type p53. In contrast, none of several known anticancer drugs showed such preference. PRIMA-1 inhibited the growth of cell lines derived from various human tumor types in a mutant p53-dependent manner. This distinguishes PRIMA-1 from known anticancer drugs and supports the idea that PRIMA-1 can serve as a lead for the development of novel therapeutic compounds.

Materials and methods

Data for 60 human tumor cell lines was extracted from the database at the Development of Therapeutic Program, NCI (see web site: http//dtp.nci.nih.gov). We selected 34 lines using the following criteria: availability of sequence information for p53 status; data on p53 protein levels; growth suppression profiles for all compounds selected for analysis; and data for at least two cell lines carrying wild-type p53 and two lines carrying mutant p53 for each cancer type. We extracted growth inhibition profiles for 44 drugs representing major mechanisms of activity: alkylating agents; topoisomerase I inhibitors; topoisomerase II inhibitors; antimitotic agents; DNA antimetabolites and DNA/RNA antimetabolites. PRIMA-1 has the designation NSC 281668 in the NCI database.

Statistical analysis was carried out using Statistica 99 Edition statistical package. The GI\(_{50}\) profiles were compared by using the Compare algorithm of the NCI database (15). GI\(_{50}\) is defined as the concentration of a tested compound that causes 50% growth inhibition and serves as an index of cytotoxicity. The obtained GI\(_{50}\) values were analyzed by the Wilcoxon matched pairs test. Cluster analysis was performed according to Ward’s method which evaluates distance between clusters through the analysis of variance by minimizing the sum of squares of any two hypothetical clusters that can be formed at each step. Ward’s amalgamation method generates more coherent clusters compared with other average linkage methods available in our Statistica 99 Edition statistical package. As a linkage distance between clusters we have chosen the Pearson correlation coefficient.
The available data on levels of different molecular targets in the 60 cell lines in NCI’s database provided us with a tool to find associations between target expression levels and sensitivity or resistance to a particular drug. We therefore used mutant p53 levels as a seed in the Compare program to search the diversity set of the NCI library. The variance parameter was set to 0.05. Analysis of GI50 patterns of all mutant p53-carrying cell lines in the database revealed several compounds in the diversity set that showed a correlation between mutant p53 expression levels and sensitivity. PRIMA-1 emerged at a ranking position of 28. The correlation between PRIMA-1 sensitivity and mutant p53 levels was relatively weak (Pearson correlation coefficient: 0.33). A search among the subset of mutant p53-carrying cell lines selected as described in Materials and methods (Table I) resulted in a ranking position of 9 for PRIMA-1. The three compounds that showed the highest correlation coefficients were NSC117281 ($r = 0.818$), NSC321206 ($r = 0.815$), and NSC150117 ($r = 0.781$). The only compound that showed a statistically significant preference for growth inhibition of mutant p53-carrying cell lines ($P = 0.04$ according to the Wilcoxon matched pairs test) was NSC117281. This compound also showed a moderate correlation between proliferation rate and sensitivity to treatment ($r = 0.4$, $P = 0.096$). Neither NSC321206 nor NSC150117 showed any statistically significant growth inhibition of mutant p53-carrying lines ($P = 0.35$ for both compounds). None of these compounds or other compounds that showed correlation between mutant p53 levels and sensitivity scored in our previous experimental screening approach that employed cells expressing mutant p53 and p53 null cells (1).

The correlation between mutant p53 expression levels and GI50 value for PRIMA-1 was statistically significant ($r = -0.75$, $P = 0.0002$; Figure 1). In contrast, there was no significant correlation between PRIMA-1 sensitivity and wild-type p53 expression levels ($r = 0.18$, $P = 0.51$). We also observed an association between levels of mutant p53 expression and cell population doubling time ($r = -0.53$, $P = 0.018$; Figure 1). Wild-type p53 expression levels did not correlate with population doubling time ($r = -0.16$, $P = 0.57$). Since cell proliferation rate correlated with mutant p53 levels, we tested whether there was any association between sensitivity to PRIMA-1 and cell population doubling time. Regression
Fig. 1. Regression analysis of the relationship between GI50 values for PRIMA-1 and p53 expression levels, p53 expression levels and cell population doubling time, and GI50 values for PRIMA-1 and cell population doubling time. Upper panel shows analysis for mutant p53-carrying cell lines and lower panel analysis for wild-type p53-carrying cell lines. p53 expression levels are shown in relative units (R.U.).

Next we examined the contribution of mutant p53 expression levels and cell growth rate to the sensitivity to 44 known anticancer agents and PRIMA-1. In other words, how unique is the observed association between mutant p53 levels and sensitivity to PRIMA-1? The results of the regression analysis are presented in Table II. Although some drugs showed a correlation between mutant p53 expression levels and sensitivity, the correlations were in general weaker than for PRIMA-1. The drug Flora could have the highest correlation coefficient with levels of mutant p53 expression after PRIMA-1 (r = -0.64, P = 0.003), but a strong association with cell population doubling time (r = 0.51, P = 0.03) was also observed. Population doubling time could be an important factor determining sensitivity of cells to treatment with drugs that interfere with DNA/RNA synthesis (Table II). Drugs that showed correlation between GI50 value and population doubling time included 5-fluorouracil, methotrexate, dichloroacetyltosone, aminopterin derivative, paclitaxel, dolastatin 10, busulfan and mitomycin C. The GI50 values correlated with population doubling time in cell lines carrying wild-type p53. Hence, rapidly proliferating cells with wild-type p53 status were more sensitive to the treatment with drugs such as thiopurine, macbecin II, PALA, dichloroacetyltosone, deoxydoxorubicin, and N,N-dibenzyl daunomycin.

Spectrum of action of PRIMA-1 in comparison to known anticancer drugs

In order to investigate the correlation between PRIMA-1 growth suppression activity and mutant p53 expression levels, we selected 34 cell lines representing different tumor types (see Materials and methods). Since PRIMA-1 reactivates mutant p53 and thus presumably requires a certain level of mutant p53 expression for its anti-tumor activity, we selected cell lines with mutant p53 levels exceeding 1 unit for further analysis. Figure 2 shows the efficiency of drugs in mutant and wild-type p53-carrying lines of each tumor type as assessed from their average GI50 values. PRIMA-1 has a statistically significant preference for mutant p53-carrying lines (P = 0.04 according to Wilcoxon matched pairs test). The same statistical analysis did not reveal any preferences in growth inhibition...
pattern for adriamycin ($P = 0.47$), methotrexate ($P = 0.35$), or paclitaxel ($P = 0.9$). Cisplatin had a significantly stronger effect on cells carrying wild-type p53 ($P = 0.08$). Similarly, 5-fluorouracil was more efficient in lines carrying wild-type p53 but the difference was not statistically significant ($P = 0.14$).

As shown in Figure 3, PRIMA-1 had a stronger growth-inhibitory effect on tumor lines harboring mutant p53 compared with wild-type p53-carrying lines in all cancer types studied. Paclitaxel was even more selective for mutant p53-expressing cells than PRIMA-1 in lines derived from ovarian and renal cancer. Methotrexate preferentially inhibited growth of mutant p53-carrying ovarian cancer lines. Adriamycin did not show any preferences towards wild type or mutant p53-carrying lines in any of the tumor types. Cisplatin showed selectivity for wild-type p53-carrying colon cancer lines and particularly melanoma lines. Finally, 5-fluorouracil was more efficient against wild-type p53-carrying cell lines derived from colon cancer and non-small cell lung cancer.

**Possible mechanisms for resistance to PRIMA-1**

By running the Compare program in a reverse manner on all cell lines carrying mutant p53, we obtained a correlation...
between levels of specific molecular targets and resistance to treatment. We found that resistance to PRIMA-1 was associated with expression of the IGF1 (insulin-like growth factor 1) receptor ($r = 0.35$) and with levels of the FER proto-oncogene tyrosine kinase ($r = 0.44$). For the other drugs tested, resistance was associated with activity of the MDR (multidrug resistance) gene and with expression of different growth factors. In non-small cell lung cancer cell lines resistance to PRIMA-1 was associated with high levels of the drug efflux pumps LRP (lung resistance protein; $r = 0.9$) and MRP (multidrug resistance protein; $r = 0.79$).

**PRIMA-1 clusters separately from known anticancer drugs**

We next asked whether PRIMA-1 could be grouped with any known anticancer drugs. Using a cluster analysis we generated a dendrogram based on the activity pattern of 43 drugs in the selected 34 cell lines. Most of the selected compounds either have been or are in clinical use, or are in clinical trials. Figure 4 shows clustering of the selected compounds in mutant p53 expressing cell lines with growth inhibition data being normalized for cell population doubling time. PRIMA-1 clustered together with the purine analogs thioguanine and thiopurine. Major groups of compounds were not as easily recognizable as in case of analysis of all 118 compounds in all cell lines in the NCI database (13). However, clustering according to the mechanism of action was obtained. Some drugs, e.g. camptothecin, formed a tight cluster while others formed groups with each other in two to three different clusters per type of agent.

**Discussion**

Tumors carrying mutant p53 are often more resistant to conventional cancer therapy (9–11). Therefore, it is important to develop novel therapeutic strategies that target mutant p53 in tumors. We have recently identified a novel mutant p53-reactivating molecule, PRIMA-1 (1). In this study, we have analyzed sensitivity to PRIMA-1 in human tumor cell lines using available information from the database at NCI. We asked to what extent PRIMA-1 would preferentially suppress growth of mutant p53-expressing lines and compared the growth inhibition pattern of PRIMA-1 with that of known anticancer drugs.

Our in silico search among 2000 compounds of the NCI diversity set for compounds that inhibit cell growth in a mutant p53-dependent manner yielded eight compounds that showed higher correlation with mutant p53 expression than PRIMA-1. However, these compounds did not score in our cellular screening assay (1). There are several possible reasons for this discrepancy. First, our cellular screening assay was designed to identify compounds that were active in the presence of mutant p53 but inactive in p53 null cells. Therefore, compounds whose activity correlates with mutant p53 levels may not necessarily score in our assay. This may also explain why PRIMA-1 did not show the best correlation between sensitivity to treatment and mutant p53 expression levels among the compounds in the diversity set.

Second, we used only one concentration of compounds in our cellular screening, i.e. 25 µM. It is obvious that compounds with GI50 values significantly higher or lower than 25 µM would not score as selective for mutant p53-expressing cells in this screening. This was apparently the case for NSC321206 and NSC150117 that scored at ranking positions 2 and 3, respectively, in our in silico search but were toxic in a mutant p53-independent manner in our cellular screening (V. Bykov, unpublished data). Their average GI50 values were 400-fold (NSC321206) and 15-fold (NSC150117) lower than that for PRIMA.

Third, it is relevant to consider the possibility that mutant p53 levels correlate with cell proliferation rate, as indicated by information in the NCI database. This is consistent with
previous studies showing that some p53 mutants have a growth stimulatory activity (16,17), which might at least in part be due to their ability to induce illegitimate expression of growth promoting genes like c-myc, NF-κB, PCNA, and others (18–21). It is conceivable that only some mutant p53 proteins stimulate cell proliferation. Therefore, the correlation between mutant p53 levels and growth suppression by NSC117281 and other compounds that showed a similar correlation could simply reflect a preferential effect on rapidly dividing cells. In contrast, sensitivity to PRIMA-1 did not correlate with cell proliferation rate. This may indicate that PRIMA-1 targets a broader range of p53 mutants than those capable of stimulating cell growth.

We observed a positive correlation between sensitivity to treatment with PRIMA-1 and levels of mutant p53 expression. This is consistent with our previous study demonstrating that PRIMA-1 reactivates mutant p53 and preferentially kills mutant p53-expressing tumor cells (Bykov et al., 2002). In contrast, our regression analysis of 44 selected anticancer agents showed a negative correlation between levels of mutant p53 and drug sensitivity in most cases. Only a few compounds showed a positive correlation, although weaker than that for PRIMA-1. For several compounds growth inhibition of tumor cells correlated positively with cell population doubling time, indicating that cell proliferation rate is a more important factor than p53 status for sensitivity to such drugs.

We did not observe any significant negative correlations between GI50 values and wild-type p53 levels, indicating that
overexpression of wild-type p53 renders cells more resistant to many drugs. A plausible explanation is that tumor cells accumulate a number of apoptosis-evading genetic changes in order to survive high levels of wild-type p53, resulting in increased drug resistance.

To compare sensitivity to PRIMA-1 with that of known anticancer drugs we selected cisplatin, 5-fluorouracil, adriamycin, paclitaxel, and methotrexate which are widely used for cancer therapy. Of these agents, cisplatin (22) and 5-fluorouracil (8) have been shown to preferentially affect cells carrying wild-type p53. Only paclitaxel has some specificity towards cells expressing mutant p53 (7). This unusual property of paclitaxel has made it an important component of frontline and adjuvant treatment in a number of chemotherapeutic regimens used in the clinic today (23). Our analysis revealed that PRIMA-1 has an overall selectivity for mutant p53-carrying tumor cell lines. In this respect PRIMA-1 is superior to paclitaxel, which also has such selectivity, but not for all cancer types studied. However, paclitaxel showed a significant selectivity for mutant p53-carrying ovarian and renal carcinoma lines. PRIMA-1 was the only agent that showed a mutant p53-selective effect on lung and colon carcinoma lines. This is of importance in view of the fact that p53 mutations occur in almost 60% of lung carcinomas and around 50% of colon carcinomas (6). A similar selectivity for mutant p53-expressing lines was observed for melanoma, although only around 10% of melanomas carry p53 mutation (6). Finally, our analysis showed that 5-fluorouracil and cisplatin preferentially inhibited the growth of lines carrying wild-type p53, in agreement with previous studies (12).

The database analysis provided several clues as to possible mechanisms of resistance against PRIMA-1. One obvious mechanism is loss of mutant p53 expression. In addition, it seems likely that increased expression of the IGF1 receptor or other receptors that mediate survival signalling could raise the apoptotic threshold and thus contribute to resistance. Common mechanisms for resistance to chemotherapy, such as expression of the MDR gene or other drug efflux pumps (24) could presumably also make cells less sensitive to PRIMA-1.

Our cluster analysis demonstrated that PRIMA-1 does not belong to any of the tight clusters of chemotherapeutic drugs formed in dendrogram. PRIMA-1 clustered with purine analogs belonging to the group of DNA antimetabolites. The clustering of that group was quite loose, indicating different mechanisms of action of drugs within the group. PRIMA-1 does not have any structural resemblance to nucleotide analogs that would favor a mechanism of activity similar to thioguanine and thiopurine. This argues that PRIMA-1 acts by mechanisms different from those of major groups of anticancer agents.

Based on our analyses of information in the NCI database,
we conclude that PRIMA-1 has preferential growth inhibitory activity on human tumor cell lines carrying mutant p53. This distinguishes PRIMA-1 from the commonly used anticancer drugs. Our results support the notion that PRIMA-1 or structural analogs of PRIMA-1 may serve as lead compounds for the development of novel anticancer drugs for more efficient treatment of tumors carrying mutant p53.

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References


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