Gain-of-Function p53 Mutations Enhance Alteration of the T-Cell Receptor following X-Irradiation, Independently of the Cell Cycle and Cell Survival

Keisuke S. Iwamoto, Terumi Mizuno, Takashi Ito, Naohiro Tsuyama, Seishi Kyoizumi, and Toshio Seyama

Department of Radiobiology, Radiation Effects Research Foundation, Hiroshima 732, Japan

Abstract

Missense mutations are by far the most common types of mutations found in p53 of human tumors, suggesting that mutant p53 proteins function either by abrogating wild-type function or by gaining new oncogenic functions. To distinguish between the dominant-negative effect and gain of new function of p53 missense mutants, we measured the ability of transfected missense mutant p53s in p53-null Jurkat cells to alter T-cell receptor (TCR) surface expression. The TCR is a key signal transduction moiety common to T lymphocytes and is one of the major sites for aberrations in T-cell leukemias/lymphomas. Three p53 mutants (248<sup>R</sup>W, 249<sup>R</sup>W, and 273<sup>R</sup>H) enhanced the frequency of TCR mutants after graded doses of X-radiation compared to null p53 parent- and wild-type p53-possessing normal lymphocytes; the parent Jurkat and normal lymphocyte showed no difference. These enhancements were not the results of a change in radiosensitivity or in G<sub>1</sub> checkpoint arrest characteristics. Therefore, the creation of this mutator phenotype by missense-type p53 mutations implies that a more direct mechanism, apart from changes of cell cycle kinetics or cell death, may be responsible for the selection of certain p53 point mutations, which eventually result in the tumorigenesis of the cell.

Introduction

Deviations from normalcy of certain gene functions important in maintenance of proper cellular integrity through, e.g., repair of damage or apoptosis of irreparably damaged cells, can direct some cells toward malignancy (1). These cells may do so by acquiring a mutator phenotype, in which nonlethal mutations accumulate as the cells continue to divide regardless of their abnormality. As mutations in certain key genes impart growth or other selective advantages to the cell, the probability of neoplastic transformation increases. p53 is found mutated in a wide variety of tumors (2). Compared to cells with wild-type p53, those with nonfunctional or abnormal p53 have been shown to be sensitive to radiation-induced mutations and resistant to radiation-induced death (3–5). The crucial determinant to these observations is the loss of function, loss of G<sub>1</sub> arrest and the ability to undergo programmed cell death, which implies that all alterations that can abrogate normal p53 function, such as deletions, nonsense mutations, frameshift mutations, exon-skipping mutations, and missense mutations, should be found with roughly equal frequency in cancers. However, most p53 mutations in human tumors are missense, suggesting that p53 may have oncogenic properties (2). The oncogenic nature can be manifested in two ways, as a dominant-negative effect (6) or as a newly gained function (7, 8). In the case of a dominant-negative effect, the mutant abrogates the function of wild-type p53; thus, there should be no difference whether a cell is homozygous, hemizygous, or heterozygous for a mutant p53, and therefore should be phenotypically indistinguishable from a p53-null cell. A number of studies has demonstrated the modulation or abrogation of wild-type p53 function by mutant p53 (6, 9). The cellular responses to DNA-damaging agents such as ionizing radiation should be similar to cases of a p53-null system; i.e., there should be a loss in the G<sub>1</sub> checkpoint arrest or the ability to undergo apoptosis. On the other hand, mutant p53s that gain new function are not well characterized. Studies have shown that mutant p53 expressed in cells lacking p53 can result in enhanced tumorigenicity, metastatic potential, and tissue invasiveness in mice or enhanced cloning efficiency in vitro (7, 10). However, little is known about the response of cells expressing only mutant p53 to ionizing radiation. Here, we show that in response to X-radiation p53-null cells develop an enhanced mutator phenotype, measured as mutant TCR<sup>3</sup> frequency, only after transfection with a p53 missense mutant. This new trait is independent of cell cycle arrest and cell survival characteristics. The possible relevance of alteration of the TCR is also discussed.

Materials and Methods

Cell Lines. Human Jurkat F-1884 cells and phytohemagglutinin-stimulated PBLs were cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 1-glutamine (2 mM), penicillin (100 units/ml), and streptomycin (100 pg/ml) in a 5% CO<sub>2</sub> humidified atmosphere. PBL cultures also contained human recombinant interleukin 2 (2 ng/ml). Cell growth was determined from the logarithmic proportions of the growth curves generated by counting cell numbers for 2 weeks.

Transfections. The Jurkat cells were stably transfected with one of three different p53 mutants by electroporation (Gene Pulser; Bio-Rad, Richmond, CA; 0.8 kV, 25 μF, time constant = 0.9 ms, in Dulbecco’s PBS). The plasmid construct was pCMVneo, into which was inserted one of the following p53 cDNAs: (a) pCS5-SN (wild type); (b) pc53-248 (arg to trp); (c) pc53-249 (arg to trp); and (d) pc53-273 (arg to his). The plasmids were a generous gift from Dr. Bert Vogelstein (The Johns Hopkins University School of Medicine Oncology Center, Baltimore, MD). The vector alone was used as control.

Following selection in 1 mg/ml G418 (Life Technologies, Inc.), individual colonies were manually picked up under microscopy and cloned a second time. The cloned transfectants were verified to contain the p53 cDNA using PCR, with primers located in exons 4 and 5. Discrimination from the genomic p53 was thus based on ampiclon size. The procedure was initially confirmed using Southern blot analysis. The transfectants are referred to as JKT-C53-SN, JKT-C53-248, JKT-C53-249, JKT-C53-273, and JKT-neo.

Materials and Methods

Sequencing. The parental Jurkat DNA was sequenced according to the Sanger dideoxy method using a double-strand DNA Cycling Sequencing System kit (Life Technologies, Inc., Grand Island, NY) per the manufacturer’s protocol.

Immunoprecipitation. Expression of the transfected p53 was confirmed by immunoprecipitation as described elsewhere (11) using PAb122 monoclonal antibody (PharMingen, San Diego, CA).

Received 5/31/96; accepted 7/16/96.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

This publication is based on research performed at the Radiation Effects Research Foundation, Hiroshima, Japan. The Radiation Effects Research Foundation is a private nonprofit foundation funded equally by the Japanese Ministry of Health and Welfare and the United States Department of Energy through the National Academy of Sciences.

To whom requests for reprints should be addressed, at Department of Radiobiology, Radiation Effects Research Foundation, 5-2 Hijiyama Park, Minami-Ku, Hiroshima 732, Japan.

This work was supported by grants from the US Department of Energy (DE-AC02-76CH00016) and the Japanese Ministry of Health and Welfare (H1-105015). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

© 1996 American Association for Cancer Research.
Flow Cytometry. Measurement of TCR/CD3 Mf has been described previously (12). Briefly, \(2 \times 10^{-6}\) log phase growing cells were incubated on ice for 30 min with phycoerythrin-labeled anti-Leu4 (CD3) and FITC-labeled anti-Hle-1 (CD45) monoclonal antibodies (Becton Dickinson, San Jose, CA). After being washed, the cells were suspended in Dulbecco’s PBS containing 1% FCS and 10 \(\mu\)g/ml propidium iodide to gate out dead cells. The cells were analyzed on a FACScan flow cytometer (Becton Dickinson). The Mf was calculated as the proportion of CD3+45+ cells in the total counted CD45+ population, as described in depth previously (12).

Irradiation. The cells were X-irradiated using a Shimadzu (WSI-250S) X-ray generator operated at 220 kVp, 8 mA, with 0.3 mm copper and 0.5 mm aluminum filtering, giving a dose rate of 2.8 Gy/min. Cells were analyzed using a FACScan 7 days following irradiation for measurement of Mf and at the given times for cell cycle kinetic analysis.

Cell Cycle Analysis. Cells were irradiated and fixed at various times afterward using methanol/acetic acid (9:1). The fixed cells were treated with RNase, stained with propidium iodide and run on a FACScan flow cytometer. The cell cycle distributions were generated using SOBR analysis of the CellFit program of the FACScan.

Cell Survival. Cells were seeded at densities ranging from 1 cell/well to 50,000 cell/well in 96-well flat-bottomed microtiter plates (Costar, Cambridge, MA) in triplicate and irradiated. Wells with and without confluent cell growth were scored. Survival and cloning efficiency were determined by calculations based on the Poisson distribution.

Statistical Analyses. Student’s t test or a previously described method (13) was used for determination of statistical significance.

Results and Discussion

Based on much accumulated data, it is evident that p53 acts as a cellular response gene to damage by exogenous agents such as ionizing radiation (3–5). The mechanisms are unclear but a popular idea is that the damage-incurred cell is dealt with by repair or eradication through p53 control of G1 arrest or apoptosis, respectively. Therefore, the loss of p53 wild-type function leads to the loss of the aforementioned cellular responses and is observed as an increase in mutation induction or cell survival following exposure to DNA-damaging agents. Our results, in contrast, show a gain of function of mutant p53 that increases mutation induction following X-irradiation, independently of cell cycle or cell survival characteristics. To explore the consequences of a mutant p53’s newly acquired function leading to the induction of a mutator phenotype, we used a sensitive flow cytometric method to detect a loss in TCR/CD3 surface expression following transfection of various mutant p53s into Jurkat cells.

The CD3-associated TCR-\(\alpha/\beta\) heterodimer is expressed on the surface of a vast majority of T cells in the peripheral blood and lymphoid organs. The TCR and CD3 are thought to form a functional unit in antigen recognition and signal transduction. The association of all of the components are essential for cell surface expression of the molecular complex (14). Consequently, inactivation of a gene encoding any protein of the TCR/CD3 complex leads to a loss of surface expression and thereby results in defective antigen recognition and cell activation. Using molecular analysis of mutant T-cell clones with defects in TCR/CD3 expression, it was determined that these losses of surface TCR/CD3 expression were caused by mutations in TCR-\(\alpha\) or \(\beta\) (12).

Normally, spontaneous Mfs range from 1 to \(6 \times 10^{-4}\) in PBLs but will rise dose dependently upon exposure to chemical and physical mutagens such as anticancer drugs and ionizing radiation (12, 15, 16). The T-cell leukemia cell line Jurkat was used because it expresses the TCR/CD3 complex on its surface at normal (compared to normal human peripheral T lymphocytes) levels. Moreover, the parental Jurkat cells were found to express no p53 protein by immunoprecipitation. Direct sequencing of the endogenous genomic p53 revealed a homozygous or hemizygous nonsense mutation in codon 196 (data not shown).

All transfectants, including the parental Jurkat cells and the vector-only clones, were measured for increases in spontaneous Mf (Table 1). The vector-only had no effect on the spontaneous Mf, which was consistently low and comparable to the parental line. Maintaining the JKT-C53-SN clones was possible for only a finite number of passages (Table 1). Fortunately, we were able to generate Mfs, although using relatively few cells; the mean value of the three clones was not significantly different from that of the control. Four JKT-C53-248 clones also showed no significant increase in spontaneous Mf over

<table>
<thead>
<tr>
<th>Table 1 Spontaneous TCR Mf and growth rates of Jurkat and p53-transfected Jurkat clones</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transfectant</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Jurkat-parent</td>
</tr>
<tr>
<td>JKT-neo</td>
</tr>
<tr>
<td>JKT-C53-SN</td>
</tr>
<tr>
<td>JKT-C53-248</td>
</tr>
<tr>
<td>JKT-C53-249</td>
</tr>
<tr>
<td>JKT-C53-273</td>
</tr>
</tbody>
</table>

*a* NG, no growth.
that of the controls. The means of four and three clones of JKT-C53-249 and JKT-C53-273, respectively, were both approximately twice those of the controls but there was no statistically significant difference.

When irradiated, the p53-null parental Jurkat responded nearly identically to the wild-type p53-possessing PBLs in the accumulation of TCR/CD3 mutants. However, the radiation-induced accumulation of TCR/CD3 mutants was enhanced in mutant p53-expressing transfectants in a dose-dependent manner when compared to the controls (Fig. 1). At all measured doses above 0 Gy, Ms of the missense mutant p53 clones were significantly higher than that of the controls. Yet, as demonstrated in Fig. 2, only the normal lymphocytes exhibited G1 checkpoint arrest at approximately 12 h postirradiation. The G1 arrest in the normal lymphocytes is not seen as a peak in the graph because the arrest at G2-M seems to precede that at G1, and thus no new cells were allowed to enter G1. The cell cycle kinetics of the Jurkats, parent and transfectants alike, were all indistinguishable, exhibiting no G1 arrest but having an abnormal, but intact G2 arrest. These results suggest that p53 control of G1 checkpoint arrest is unrelated to the accumulation of radiation-induced TCR/CD3 mutants, and that the gain of function of mutant p53 leading to a mutator phenotype is possibly via a more direct mechanism than the prevention of effecting repair by stopping cell cycle progression.

Similarly, doubling times of the transfectants showed that with the exception of the wild-type transfectants, parents, and all other transfectants, doubling rates were not statistically different (P > 0.1), with an average of 29 h/cycle (Table 1). In the Jurkat cell, where no p53 is expressed, the type of mutant p53 had no significant effect on the growth characteristics but wild-type p53 was able to suppress the growth. These results are consistent with the concept that the growth rate is dependent on wild-type p53 and are further support for the idea that the mutator phenotype induced by the mutant p53s are not due to cell kinetic changes.

There was no statistically significant difference in the radiation-induced Ms among the clones carrying different p53 mutations. This latter observation is a curious result because one would assume that gain of function mutations should be relatively specific. Notably, three hotspot point mutations were used in this study; other missense mutations may not have had such effects. Furthermore, there may exist real differences among the tested mutants as the mean values would seem to indicate at the higher doses, but the variability may have been too large for statistical differences to be detected.

Next, we questioned whether the mechanism may be explained by changes in radiosensitivities. Therefore, one hypothesis was that the mutant p53-transfected Jurkat clones exhibited high TCR/CD3-induced Mf because the mutant p53 somehow prevented death of damaged cells. However, as shown in Fig. 3, there are no significant differences in radiation sensitivities. The parental and transfected Jurkat clones were all similarly radiosensitive (D0 = 0.65 Gy) and agree with measurements made by others on Jurkat cells (17). It is interesting that Jurkat cells are relatively radiosensitive in light of the fact that a number of articles report increases in radioresistance.
following loss of p53 from cells, including those of hematopoietic lineage, explained as a loss in p53-dependent radiation-induced apoptosis leading to survival of cells that should have been directed to commit suicide (4). Because our results show no increase in survival following transfection of mutant p53 into the p53-null Jurkat cells, enhanced induction of Mf in those same transfectants cannot be attributed to a decreased loss by death of TCR/CD3 mutants.

The independence of the radiation-induced increases in TCR/CD3 Mf to cell cycle arrest and cell survival observed in the present study supports the idea of gain of function mutations in p53 because G1 checkpoint arrest and radiation-induced apoptosis are functions of wild-type p53, and their losses are identified with the loss of wild-type p53 function. That is, these two responses are associated with the recessive, tumor suppressor nature of p53 and not the dominant oncogenic nature.

This oncogenic trait of p53 has been suspected for years; Lane and Benchimol (18) proposed a hypothesis for p53's gain of function based partly on early work showing increased tumorigenicity (19, 20) and metastatic potential (21) when p53 protein, later recognized to be mutant forms, was expressed in cells. In recent years, it has become evident that there is a clear bias for human tumors to possess missense mutations in p53 (2). In terms of mechanism, studies have suggested some possibilities. For example, some mutant p53 proteins can enhance reporter gene expression, inferring that mutants may gain a function that can alter gene expression (7).

The precise role of p53 in radiation carcinogenesis is unclear. In mice, an increased susceptibility to radiation-induced tumors has been observed in transgenic animals carrying mutant p53. Unfortunately, p53-null mice were not included in those experiments; therefore, gain of function versus loss of function could not be compared (22). Our results clearly point to the relevance of p53 missense mutations in having a truly dominant role in fostering a mutator phenotype that could favor cellular progression into neoplasia.

This fostering of a mutator phenotype could help to explain not only the prevalence of missense-type mutations of p53 in human tumors but also the existence of hotspot missense mutations. Moreover, because such hotspot mutations are often associated with tissue type, interesting new effects of respective p53 mutants may delineate tissue-specific carcinogenesis. Whether alterations of the TCR/CD3 complex caused by radiation and missense mutant p53 are crucial in influencing the neoplastic fate of a lymphocyte is unknown.

We have shown that the gained function, in this case, has little to do with the general cellular responses of G1 arrest and cell death to ionizing radiation. Thus, the described mutator phenotype may not apply to all loci. Certainly, the TCR/CD3 complex is an important tissue-specific moiety that plays multiple roles in the T lymphocyte. It is interesting that the most consistent cytogenetic anomaly in adult T-cell leukemia/lymphoma and T-ALL is found at the TCR gene (23, 24). It is noteworthy that recently, based on the evaluation of 51 adult T-cell leukemia/lymphoma patients, it was postulated that p53 alterations represent one of the genetic changes responsible for progression of the disease (25). In T-ALL, p53 mutations are frequent in cancer that has relapsed, suggesting an important role in the progression of T-ALL. Additionally, relapse patients with p53 mutations are often refractory to induction of remission by additional therapy with an overall decreased duration of survival and a 3.8-fold increased risk of death compared to patients without p53 mutations (26). Our results may imply that p53 missense mutations per se are important when considering the cellular responses to oncotherapeutic agents.

Acknowledgments

We are grateful to Shioso Fujii, Norie Ishii, Chiyoe Saito, and Tomoko Shinohara for technical support and Drs. Yoichiro Kusunoki, Nori Nakamura, Donald R. Harkness, and Mortimer L. Mendelsohn for encouragement, discussions, and critical reading of the manuscript.

References

10. Hsiao, M., Low, J., Dorn, E., Ku, D., Pattengale, P., Yeargin, J., and Haas, M. Gain-of-function mutations of the p53 gene induce hyperproliferation, tumorigenicity and metastatic potential (21) when p53 protein, later recognized to be mutant forms, was expressed in cells. In recent years, it has become evident that there is a clear bias for human tumors to possess missense mutations in p53 (2). In terms of mechanism, studies have suggested some possibilities. For example, some mutant p53 proteins can enhance reporter gene expression, inferring that mutants may gain a function that can alter gene expression (7).

Gain-of-Function p53 Mutations Enhance Alteration of the T-Cell Receptor following X-Irradiation, Independently of the Cell Cycle and Cell Survival

Keisuke S. Iwamoto, Terumi Mizuno, Takashi Ito, et al.

Cancer Res 1996;56:3862-3865.

Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/56/17/3862

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.