

## BRIEF COMMUNICATION

### Frequency of p53 Mutations in Hepatocellular Carcinomas From Atomic Bomb Survivors

Keisuke S. Iwamoto,  
Terumi Mizuno, Shoji Tokuoka,  
Kiyohiko Mabuchi,  
Toshio Seyama\*

The mechanism of liver carcinogenesis is unclear, although it is believed to involve multiple endogenous and exogenous genetic alterations. One form of liver cancer, hepatocellular carcinoma (HCC), is particularly common in Africa and Asia, including Japan (1). Studies have demonstrated a strong association between HCC and hepatitis B and C virus infections (2). The HCC cells also exhibit frequent allelic losses on specific chromosomal arms in their nuclei, particularly losses in the single most commonly mutated gene in human cancers, the tumor suppressor gene p53 (also known as TP53) (3).

Because of the relatively long latency period, the mechanistic role of ionizing radiation in the dose-dependent increased frequency of HCC among the atomic bomb survivors of Hiroshima and Nagasaki is not clear (4). To shed some light on the molecular events that may, in part, help to explain the heightened risk of atomic bomb-induced HCC, we have analyzed the p53 gene in the HCC tissue samples from 120 survivors of the atomic bomb explosions who were exposed to various doses of radiation ranging from 0 to 1569 mSv (liver dose).

The tissues were procured from 1952 through 1989. All specimens were formalin fixed and paraffin embedded at the time of autopsy. DNA was extracted separately from areas of each sample containing normal or tumor tissues and amplified by polymerase chain reaction

(5). The mutational-hotspot exons 5, 6, 7, and 8 of the p53 gene were initially screened by the single-strand conformation polymorphism assay for mutations and sequenced by the dideoxy method. Sequencing was done in both the sense and antisense directions, and only mutations that were verified in both directions were counted. All samples were analyzed after blinding as to exposure dose, city of exposure, sex, and age.

The samples were divided into the following four dose groups: 0–4, 5–249, 250–499, and greater than or equal to 500 mSv. The number of samples in each group is shown in Fig. 1. There was no difference among the four groups with respect to the characteristics of the detected mutations. In all groups, roughly 70% of the point mutations were heterozygous and 30% were homozygous (most likely, hemizygous with deletion of one allele). Restriction fragment length polymorphism analysis demonstrated loss of heterozygosity in 32% of the informative samples. Seventy-eight percent of the point mutations detected were GC to AT transitions, with only 13% of these at CpG sites. Most (74%) of the mutations were of the missense type, and 21% were silent mutations; however, 89% of the samples with silent mutations also had a missense mutation. Finally, there was no remarkable difference with respect to dose in the location of the mutations along the p53 gene.

As controls, the nontumor tissues from the 0–4, 249–499, and greater than or equal to 500 mSv groups were analyzed and shown to have p53 point mutations at frequencies of 45%, 44%, and 20%, respectively. The results were verified by two independent polymerase chain reaction amplifications from the stock (unamplified) DNA. The nonexistence of a significant dose dependence and the high frequency of mutations is provocative. A number of studies (6–8) have reported high frequencies of p53 abnormalities in nontumor liver tissues from patients with HCC. The enrichment of cells with p53 mutations in the nontumor tissues is probably caused by selective proliferation of hepatocytes with p53 mutations plus characteristic liver regenerative responses to cell loss or injury, with no concomitant increase in apoptosis. This view is supported by

data demonstrating the development of focal hepatocyte nodules in p53-transgenic rats (9). Furthermore, the low proportion of point mutations in the greater than or equal to 500-mSv group of nontumor samples may imply that irradiated precancerous cells with mutations either died or progressed to HCC.

In contrast to the nontumor tissues, there was a statistically significant dose–response relationship in the percent of HCC samples harboring a p53 point mutation in the tumor tissues (Fig. 1; logistic regression method applied to the ungrouped data gave a logistic slope of .00163 and a 95% confidence interval of .00042–.00322). The background frequency of 46% that we found is similar to the frequency in the general Japanese population reported by Oda et al. (3). It is striking that there was a dose–response relationship for point mutations but not for deletions, which are one of the major types of DNA damage thought to be caused by ionizing radiation (10). One consideration is that cells with deletions could have died early, leading to a negative selection of deletion-type damage.

The dose-dependent enrichment of cells with p53 mutations in the tumors is probably caused by expansion of cells with p53 mutations plus mutations in other genes that allow unregulated growth. The direct radiation target is more likely to be a gene that is changed into a mutator by a radiation-induced mutation. The induction of a mutator gene would be expected to increase with dose and would allow a single cell or its progeny to accumulate multiple mutations necessary for the conversion of a normal to a cancer cell.

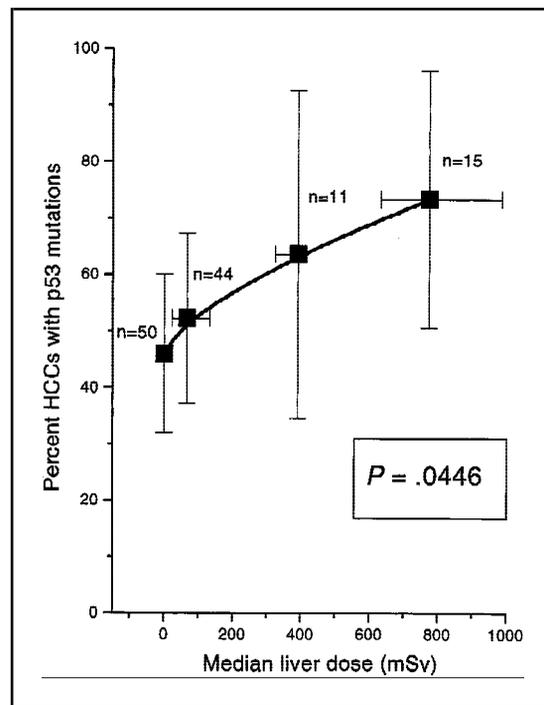
In addition, the dose-dependent rise in tumor p53 mutations and the dose-independent existence of nontumor tis-

\*Affiliations of authors: K. S. Iwamoto, T. Mizuno, T. Seyama (Department of Radiobiology), S. Tokuoka, K. Mabuchi (Department of Epidemiology), Radiation Effects Research Foundation, Hiroshima, Japan.

Correspondence to: Toshio Seyama, M.D., Department of Radiobiology, Radiation Effects Research Foundation, 5–2 Hijiyama Park, Minamiku, Hiroshima 732 Japan. E-mail: seyama@rerf.or.jp

See "Notes" following "References."

**Fig. 1.** Percent of hepatocellular carcinoma (HCC) samples with p53 mutations as a function of the dose of radiation from the atomic bomb to the liver. Statistical analysis was applied to the ungrouped data. Error bars for the percent HCCs with p53 mutations denote two standard errors, and the error bars for the median liver dose denote the 25th and 75th quartiles of the dose-grouped data. *P* value is two-sided.



such p53 mutations also suggest that irradiation of noncancer cells with pre-existing mutations may have increased the likelihood of progression to cancer, perhaps by a mechanism similar to the one proposed above regarding creation of a mutator gene. An analogous situation to this supposition is found in the study by Cha et al. (11) who demonstrated that *N*-nitroso-*N*-methylurea exposure was not directly responsible for the mutations in the H-ras proto-oncogene that are frequently found in *N*-nitroso-*N*-methylurea-induced rat mammary tumors (and, in fact, that the H-ras mutations were shown to pre-exist rather than be generated at a later time). Further support is found from p53-knockout mice that demonstrate enhanced HCC development after exposure to liver carcinogens (12). Such studies (13) as well as ours emphasize the importance of conditional mutations whose oncogenic potentials are realized after exposure to a carcinogen and a concomitant damage of another gene. Thus, one would expect a dose-dependent rise in mutations in the tumor but not in the nontumor tissues.

Future studies in the survivor population on the associations of p53 muta-

tions with hepatitis B and C virus infections (which are found frequently in the Japanese HCC population), which lead to chronic cycles of cell loss, regeneration, and damage, should provide clearer clues to the etiology of radiation-induced human liver cancers.

## References

- (1) Di Bisceglie AM, Rustgi VK, Hoofnagle JH, Dusheiko GM, Lotze MT. NIH conference. Hepatocellular carcinoma. *Ann Intern Med* 1988;108:390-401.
- (2) Tanaka K, Hirohata T, Koga S, Sugimachi K, Kanematsu T, Ohryohji F, et al. Hepatitis C and hepatitis B in the etiology of hepatocellular carcinoma in the Japanese population. *Cancer Res* 1991;51:2842-7.
- (3) Oda T, Tsuda H, Scarpa A, Sakamoto M, Hirohashi S. p53 gene mutation spectrum in hepatocellular carcinoma. *Cancer Res* 1992; 52:6358-64.
- (4) Thompson DE, Mabuchi K, Ron E, Soda M, Tokunaga M, Ochikubo S, et al. Cancer incidence in atomic bomb survivors. Part II: solid tumors, 1958-1987 [published erratum appears in *Radiat Res* 1994;139:129]. *Radiat Res* 1994;137(2 Suppl):S17-S67.
- (5) Iwamoto KS, Mizuno T, Ito T, Akiyama M, Takeichi N, Mabuchi K, et al. Feasibility of using decades-old archival tissues in molecular oncology/epidemiology. *Am J Pathol* 1996;149:399-406.
- (6) Livni N, Eid A, Ilan Y, Rivkind A, Rosen-

mann E, Blendis LM, et al. p53 expression in patients with cirrhosis with and without hepatocellular carcinoma. *Cancer* 1995;75: 2420-6.

- (7) Kishimoto Y, Shiota G, Kamisaki Y, Wada K, Nakamoto K, Yamawaki M, et al. Loss of the tumor suppressor p53 gene at the liver cirrhosis stage in Japanese patients with hepatocellular carcinoma. *Oncology* 1997;54: 304-10.
- (8) Iwamoto KS, Mizuno T, Kurata A, Masuzawa M, Mori T, Seyama T. Multiple, unique and common p53 mutations in a thorotrast recipient with four primary cancers. *Hum Pathol* 1998;29:412-6.
- (9) Hully JR, Su Y, Lohse JK, Griep AE, Sattler CA, Haas MJ, et al. Transgenic hepatocarcinogenesis in the rat. *Am J Pathol* 1994;145: 386-97.
- (10) Goodhead DT. Initial events in the cellular effects of ionizing radiations: clustered damage in DNA. *Int J Radiat Biol* 1994;65:7-17.
- (11) Cha RS, Thilly WG, Zarbl H. *N*-Nitroso-*N*-methylurea-induced rat mammary tumors arise from cells with preexisting oncogenic Hras1 gene mutations. *Proc Natl Acad Sci U S A* 1994;91:3749-53.
- (12) Yin L, Ghebranious N, Chakraborty S, Sheehan CE, Ilic Z, Sell S. Control of mouse hepatocyte proliferation and ploidy by p53 and p53ser246 mutation *in vivo*. *Hepatology* 1998;27:73-80.
- (13) Cha RS, Guerra L, Thilly WG, Zarbl H. Hras-1 oncogene mutations in mammary epithelial cells do not contribute to initiation of spontaneous mammary tumorigenesis in rats. *Carcinogenesis* 1996;17:2519-24.

## Notes

This study was made possible by the cooperative efforts of hospitals throughout the cities of Hiroshima and Nagasaki. We thank Drs. Masayoshi Tokunaga, Toshiyuki Fukuhara, Masami Yamamoto, Hideo Itakura, Takayoshi Ikeda, Masao Kishikawa, Yasuyuki Fujita, and Nori Nakamura for the collection and pathological reviews of the tissues and/or for their many helpful suggestions. We also thank Shiho Fujii, Norie Ishii, Chiyoe Saito, and Tomoko Shinohara for technical support; Mutsumi Mizuno and Chiyako Ohmoto for tissue preparation; and Dr. John B. Cologne and Sachiyo Funamoto for statistical analyses.

This investigation using human archival tissue samples was approved by an institutional review board.

This publication is based on research performed at the Radiation Effects Research Foundation (RERF), Hiroshima and Nagasaki, Japan. RERF 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.

Manuscript received October 14, 1997; revised May 13, 1998; accepted May 20, 1998.