SHORT COMMUNICATION

Infrequent alterations of the p53 gene in rat skin cancers induced by ionizing radiation

Yi Jin1,3, Fredric J. Burns2, Seymour J. Garte2 and Steve Hosselet2

1Institute of Cancer Research, Columbia University, 701 West 168th Street, New York, NY 10032 and 2Institute of Environmental Medicine, New York University Medical Center, 550 First Avenue, New York, NY 10016, USA
3To whom correspondence should be addressed

Radiation carcinogenesis almost certainly involves multiple genetic alterations. Identification of such genetic alterations would provide information to help understand better the molecular mechanism of radiation carcinogenesis. The energy released by ionizing radiation has the potential to produce DNA strand breaks, major gene deletions or rearrangements, and other base damages. Alterations of the p53 gene, a common tumour suppressor gene altered in human cancers, were examined in radiation-induced rat skin cancers. Genomic DNA from a total of 33 rat skin cancers induced by ionizing radiation was examined by Southern blot hybridization for abnormal restriction fragment patterns in the p53 gene. An abnormal p53 restriction pattern was found in one of 16 cancers induced by electron radiation and in one of nine cancers induced by neon ions. The genomic DNA from representative cancers, including those with an abnormal restriction pattern, was further examined by polymerase chain reaction amplification and direct sequencing in exons 5–8 of the p53 gene. The results showed that one restriction fragment length polymorphism (RFLP)-positive cancer induced by electron radiation had a partial gene deletion which was defined approximately between exons 2–8, while none of the other cancers showed sequence changes. Our results indicate that the alterations in the critical binding region of the p53 gene are infrequent in rat skin cancers induced by either electron or neon ion radiation.

Radiation carcinogenesis may involve multiple genetic lesions that provide one cell with a selective growth advantage over other cells and drive its clonal growth (1–4). Identification of such genetic alterations would provide information to help understand better the molecular mechanism of radiation carcinogenesis. Rat skin cancers induced by ionizing radiation have been used as a model system to identify genetic lesions in radiation carcinogenesis (5,6). Early studies have found a high frequency of the amplification of c-myc oncogene in electron radiation-induced rat skin cancers (7). The amplification was correlated with both the size and growth rate of the cancers (8). Examination by in situ hybridization further indicated that c-myc amplification was cell-specific within a radiation induced carcinoma (9). However, it was later found that such amplification was infrequent in rat skin cancers induced by high linear energy transfer (LET) radiation in the form of neon ions (10), suggesting that c-myc amplification might be LET dependent. On the other hand, the possibility could not be ruled out that a gene other than c-myc might also be involved in carcinogenesis of rat skin.

Recent studies have shown that alterations in tumour suppressor genes represent critical genetic lesions in carcinogenesis (11,12). The two most commonly studied tumour suppressor genes, p53 and RB, have been widely examined for their role in carcinogenesis. Alterations of the p53 and RB genes, including point mutation, gene deletion or rearrangement, have been observed in a variety of human cancers (13–18). Alteration of the p53 gene has been suggested to interfere with DNA damage checkpoint function of the gene, and to allow cells to continue into S phase of the cell cycle without repairing DNA damage (19).

The purpose of this research was to determine whether alterations of the p53 gene occur in rat skin cancers induced by two types of ionizing radiation, including electron and neon ions radiation and, if so, to determine the molecular nature of the alterations by polymerase chain reaction (PCR) amplification and DNA sequence analyses.

We induced rat skin tumours by irradiating male Sprague—Dawley rats at 29 days of age. Two groups of 100 rats were irradiated with 25 keV/μm neon ions or 0.34 keV/μm electrons respectively. The doses ranged from 2 to 20 Gy for both types of radiation. Rat skin tumours were observed 12 weeks after irradiation and thereafter. The tumours were excised and stored at −80°C. Part of each tumour was prepared for histological examination. The average diameter of the tumours at the time of excision was 2.86 cm (SD = 1.59) for electron induced tumours, and 3.86 cm (SD = 2.61) for neon ion induced tumours. The types of tumours included squamous cell carcinomas, basal cell carcinomas, sarcomas, sebaceous cell carcinomas and fibromas.

We first analyzed genomic DNA from 33 tumours induced by the two types of radiation for putative abnormal restriction patterns in the p53 gene. DNA was extracted from frozen tissues by standard methods (20), and followed by digestion with the restriction enzymes, EcoRI or HindIII respectively. Restriction fragments were separated on 0.8% agarose gels and blotted onto nylon membranes. The blots were then hybridized to a human p53 cDNA fragment (Oncogene Science, Uniondale, NY) by applying a Genius™ kit, a nonradioactive labeling and detection system (Boehringer Mannheim, Uniondale, NY) by applying a Genius™ kit, a nonradioactive labeling and detection system (Boehringer Mannheim, Indianapolis, IN).

Since a high frequency of mutation has been reported in exons 5–8 of the p53 gene in human cancers, the exons of the p53 gene in selected tumours were examined by PCR amplification and direct DNA sequencing. Primers for each
cDNA digested by EcoRI were hybridized to the 18 kb EcoRl cDNA. Three fragments were probed to the genome fragment (23), which suggested that the 18 kb fragment represents a result from Southern blot hybridization for genomic DNA of the p53 gene, and the small fragment probably from the p53 pseudogene. In panel B, DNA from eight tumours was digested with HindIII and hybridized to the same probe. Three HindIII fragments (5.1, 4.2 and 3.5 kb) were seen in a positive control. Cancer 157 again showed an abnormal restriction pattern, in which the 5.1 kb fragment was replaced by two new fragments of 10 and 6 kb. The fact that cancer 157 showed abnormal restriction patterns in two restriction enzyme digestions implies that an alteration of the p53 gene might occur in this cancer. In addition, cancers 106, 134 and 156 showed only one HindIII fragment (5.1 kb); the implication of this has not been concluded. The blots were stripped and rehybridized to a plasmid into which the p53 cDNA was inserted. The result confirmed that all restriction fragments observed in Southern analyses of the cancer DNAs were derived from the rat p53 gene (data not shown).

By similar analyses we found that one out of nine neon ion-induced rat skin cancers contained abnormal restriction patterns. In cancer 353, the 18 kb fragment generated by EcoRI digestion was replaced by four new fragments with sizes of 5.5, 4.7, 4 and 3 kb. The intensity of the 4 kb fragment showed much more strongly than the others (data not shown). To determine whether the abnormal restriction patterns seen in Southern blot hybridizations possibly resulted from population polymorphism in the rat p53 gene, DNA from 12 normal rat kidneys was digested with EcoRI and HindIII, respectively, and then probed with the same probe. No restriction fragment length polymorphism (RFLP) pattern was observed in any of the DNA examined (data not shown).

In order to determine the molecular nature of the abnormal restriction patterns of the p53 gene seen in several rat skin tumours, we examined five tumours more closely by PCR amplification and DNA sequencing. The PCR examination for all exons of the p53 gene are summarized in Table II. Our results showed that cancer 157 lost exons 2–8 of the gene. The four other cancers including cancer 353 showed consistently normal four exons. The sequencing data did not reveal any sequence changes in exons 5–8 for cancer 353. Figure 2 shows the results of PCR examination at exons 7 and 8. A pair of primers (P7-1 and P8-2 in Table I) was used to amplify the exons. Two amplified fragments, ~560 and 250 bp, were observed in positive control and in three cancers: 135, 333 and 353. Only a small fragment (250 bp) was observed in cancer 157. The fact that the size of the larger fragment was consistent with the exon size from the cDNA data (26) indicates that the large fragment was derived from the functional p53 gene, and the small fragment probably from the p53 pseudogene. This was confirmed by sequencing the large fragment of the control and other cancers (data not shown). The absence of exons 2–8 in cancer 157 in PCR examinations suggests a possible internal deletion of the gene in the cancer. The breakpoints have not been precisely determined. However, they are probably located between exon 1 and exon 2, and the breakpoints have not been precisely determined. However, they are probably located between exon 1 and exon 2, and between exon 8 and exon 9 based on the PCR amplification. It is unclear what the other two fragments (4.2 and 2.0 kb) represent. Since two p53 pseudogenes have been found in rat genomic DNA (24,25), it is possible that these two fragments are derived from the pseudogenes. In panel B, DNA from eight tumours was digested with HindIII and hybridized to the same probe. Three HindIII fragments (5.1, 4.2 and 3.5 kb) were seen in a positive control. Cancer 157 again showed an abnormal restriction pattern, in which the 5.1 kb fragment was replaced by two new fragments of 10 and 6 kb. The fact that cancer 157 showed abnormal restriction patterns in two restriction enzyme digestions implies that an alteration of the p53 gene might occur in this cancer. In addition, cancers 106, 134 and 156 showed only one HindIII fragment (5.1 kb); the implication of this has not been concluded. The blots were stripped and rehybridized to a plasmid into which the p53 cDNA was inserted. The result confirmed that all restriction fragments observed in Southern analyses of the cancer DNAs were derived from the rat p53 gene (data not shown).

By similar analyses we found that one out of nine neon ion-induced rat skin cancers contained abnormal restriction patterns. In cancer 353, the 18 kb fragment generated by EcoRI digestion was replaced by four new fragments with sizes of 5.5, 4.7, 4 and 3 kb. The intensity of the 4 kb fragment showed much more strongly than the others (data not shown). To determine whether the abnormal restriction patterns seen in Southern blot hybridizations possibly resulted from population polymorphism in the rat p53 gene, DNA from 12 normal rat kidneys was digested with EcoRI and HindIII, respectively, and then probed with the same probe. No restriction fragment length polymorphism (RFLP) pattern was observed in any of the DNA examined (data not shown).

In order to determine the molecular nature of the abnormal restriction patterns of the p53 gene seen in several rat skin tumours, we examined five tumours more closely by PCR amplification and DNA sequencing. The PCR examination for all exons of the p53 gene are summarized in Table II. Our results showed that cancer 157 lost exons 2–8 of the gene. The four other cancers including cancer 353 showed consistently normal four exons. The sequencing data did not reveal any sequence changes in exons 5–8 for cancer 353. Figure 2 shows the results of PCR examination at exons 7 and 8. A pair of primers (P7-1 and P8-2 in Table I) was used to amplify the exons. Two amplified fragments, ~560 and 250 bp, were observed in positive control and in three cancers: 135, 333 and 353. Only a small fragment (250 bp) was observed in cancer 157. The fact that the size of the larger fragment was consistent with the exon size from the cDNA data (26) indicates that the large fragment was derived from the functional p53 gene, and the small fragment probably from the p53 pseudogene. This was confirmed by sequencing the large fragment of the control and other cancers (data not shown). The absence of exons 2–8 in cancer 157 in PCR examinations suggests a possible internal deletion of the gene in the cancer. The breakpoints have not been precisely determined. However, they are probably located between exon 1 and exon 2, and between exon 8 and exon 9 based on the PCR amplification. It is interesting that our PCR and DNA sequencing examination did not find any sequence changes which could explain the abnormal restriction pattern seen in cancer 353. The nature of the abnormal restriction pattern needs to be determined further. One speculation is that the abnormal restriction pattern of the p53 gene might be caused by a gene rearrangement within an intron region. Evidence for intrinsic point mutations

---

**Table I. Sequences of the primers used for PCR amplification and direct DNA sequencing in the p53 gene**

<table>
<thead>
<tr>
<th>Primer</th>
<th>Exon no.</th>
<th>Upstream sequence</th>
<th>Downstream sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1-1</td>
<td>1</td>
<td>5'-GGG TCT GCT GGG ATT GGG ACT-3'</td>
<td>5'-GGA CTC ATC AGC CCT GCC AGC CCT TTC-3'</td>
</tr>
<tr>
<td>P1-2</td>
<td>1</td>
<td>5'-GGG TCT GCT GGG ATT GGG ACT-3'</td>
<td>5'-GGA CTC ATC AGC CCT GCC AGC CCT TTC-3'</td>
</tr>
<tr>
<td>P2-1</td>
<td>2,3,4</td>
<td>5'-AGC GAT CAT CTC CAT TCG TCG GA-3'</td>
<td>5'-AGC GAT CAT CTC CAT TCG TCG GA-3'</td>
</tr>
<tr>
<td>P2-2</td>
<td>2,3,4</td>
<td>5'-AGC GAT CAT CTC CAT TCG TCG GA-3'</td>
<td>5'-AGC GAT CAT CTC CAT TCG TCG GA-3'</td>
</tr>
<tr>
<td>P3-1*</td>
<td>5,6</td>
<td>5'-GAT TCT TCT TCC TCT CCT AC-3'</td>
<td>5'-GAT TCT TCT TCC TCT CCT AC-3'</td>
</tr>
<tr>
<td>P4-2</td>
<td>5,6</td>
<td>5'-GAT TCT TCT TCC TCT CCT AC-3'</td>
<td>5'-GAT TCT TCT TCC TCT CCT AC-3'</td>
</tr>
<tr>
<td>P5-1</td>
<td>7</td>
<td>5'-TAT ACC ACT CAT CAC TAC AAG-3'</td>
<td>5'-TAT ACC ACT CAT CAC TAC AAG-3'</td>
</tr>
<tr>
<td>P7-1</td>
<td>8.5</td>
<td>5'-TGG GAA TCT TCT GGG ACG GG-3'</td>
<td>5'-TGG GAA TCT TCT GGG ACG GG-3'</td>
</tr>
<tr>
<td>P9-1</td>
<td>9</td>
<td>5'-CA CTC CCC AGC ACG ACA AGC-3'</td>
<td>5'-CA CTC CCC AGC ACG ACA AGC-3'</td>
</tr>
<tr>
<td>P10-1</td>
<td>9</td>
<td>5'-ATC CGT GGG CGT GAG CCC TTC-3'</td>
<td>5'-ATC CGT GGG CGT GAG CCC TTC-3'</td>
</tr>
<tr>
<td>P11-1</td>
<td>10</td>
<td>5'-TCC TCT CCT TCT TCT TAC TCC-3'</td>
<td>5'-TCC TCT CCT TCT TCT TAC TCC-3'</td>
</tr>
</tbody>
</table>

Primers with asterisks indicate that its sequence is in the intron region of the gene; others are in the exon region.

**Table II. Summary of the PCR examination of exons in the p53 gene for five rat skin cancers induced by electron (135, 157) and neon ion radiation (333, 353 and 362)**

<table>
<thead>
<tr>
<th>Exon no.</th>
<th>135</th>
<th>157</th>
<th>333</th>
<th>353</th>
<th>362</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2,3,4</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5,6</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

-, Samples showed missing fragments, +, samples showed same pattern as control.
Alterations in rat skin cancers

Fig. 1. Southern analyses of RFLP of the p53 gene for rat skin cancers induced by electron radiation (LET = 0.34 keV/μm). The genomic DNA from the cancers was digested with EcoRI (A) or HindIII (B), and then probed to a human p53 cDNA. NR, normal rat kidney DNA as control; BCC, basal cell carcinoma; SCC, squamous cell carcinoma; SAR, sarcoma.

Fig. 2. PCR examination of exons 7-8 in the p53 gene. The DNA fragments amplified by applying primers P7-1 and P8-2 were separated on 2% agarose gel. Lanes 1 and 2 are molecular weight markers. Lane 3 is a control DNA; lanes 4-7 are DNA from cancers 135, 157, 333 and 353. The 550 kb fragments were amplified from the functional gene and the 250 kb fragments were amplified from the pseudogenes.

of the p53 gene has been reported in the genomic DNA of small cell lung cancer cell lines, and are considered to be an alternative mechanism for p53 inactivation (27). The presence of some p53 introns appears to be critical for the regulation of this gene in vivo (28). The flanking sequences of the p53 gene could also be a target region for ionizing radiation. Since the 18 kb fragment from EcoRI digestion was reported to contain the sequence corresponding to all the p53 cDNA sequences (23), and the rat p53 gene has been estimated at 12 kb (24), it is possible that the abnormal restriction pattern seen in cancer 353 might originate from restriction site change in the flanking regions.

Overall, our results demonstrate that the alteration of the p53 gene was an infrequent event in rat skin cancers induced by two types of ionizing radiation. In fact, alterations of the p53 gene in radiation-induced cancers have not been reported as frequently as in cancers induced by chemicals, viral agents, or UV radiation in humans and animals. In one report, alterations of the p53 gene, including two total gene deletions, were found in seven of 19 radon associated lung cancers of uranium miners (29). However, the role of the p53 gene in radiation carcinogenesis is far from defined. Thraves et al. (30) have observed an absence of p53 mutations in malignant human epithelial cell transformed by high LET radiations in the form of fission neutrons.

Because of the multiplicity of target genes that may be involved in cancer progression, important alterations may be missed when focusing on only one gene. There are literally...
hundreds of genes required to maintain the stability of the genome in somatic cells, including genes involved in DNA synthesis, DNA repair, signal transduction, mitosis and the cell cycle. Alterations in any of these genes could result in genetic instability and contribute to carcinogenesis (31,32). If the population of potential gene targets for radiation carcinogenesis in somatic cells is large, it would not be surprising that deletions and rearrangements in specific genes, like p53, are found infrequently in the cancers induced by ionizing radiation.

A LET effect on oncogene activation has been observed in mouse thymomas, where the pattern of ras gene activation differs between high and low LET radiation (33). The pattern of c-myc amplification detected in rat skin cancers was different for neon ions and electron radiation, where amplification of the c-myc oncogene was frequently found in large, late-stage carcinomas of rat skin induced by electron radiation but not in cancers induced by neon ions (7). The difference in c-myc activation between high- and low-LET radiation has given credence to the idea that high and low LET radiation have different effects on carcinogenesis (23,24). In particular, the pattern of p53 dependence of the restriction pattern in the p53 gene in rat skin cancers.

Acknowledgements

The authors gratefully acknowledge the technical assistance provided by Ms Betty Skocik and Mr Greg Dudas. This work was supported in part by grant DOE ER 60539 and the American Cancer Society.

References


Received on September 27, 1995; revised on December 20, 1995; accepted on January 17, 1996.