Multigene Deletions in Lung Adenocarcinomas from Irradiated and Control Mice

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Abstract. K-*ras* codon 12 point mutations, *mRb* and *p53* gene deletions were examined in tissues from 120 normal lungs and lung adenocarcinomas that were Formalin-treated and paraffin-embedded 25 years ago. The results showed that 12 of 60 (20%) lung adenocarcinomas had *mRb* deletions. All lung adenocarcinomas that were initially found bearing deleted *mRb* had *p53* deletions (15 of 15; 100%). A significantly higher mutation frequency for K-*ras* codon 12 point mutations was also found in the lung adenocarcinomas from mice exposed to 24 once-weekly neutron irradiation (10 of 10; 100%) compared with those exposed to 24 or 60 once-weekly γ-ray doses (5 of 10; 50%). Our data suggested that *p53* and K-*ras* gene alterations were two contributory factors responsible for the increased incidence of lung adenocarcinoma in $B6CF_1$ male mice exposed to protracted neutron radiation.

INTRODUCTION

Results presented here are derived from a study of 40,000 $B6CF_1$ hybrid mice (C57BL/6 female × BALB/c male) conducted at Argonne National Laboratory from 1971 to 1986 in an effort to determine the effects of acute and chronic radiation injury. The experiments were performed by testing many different aspects of radiation effects, including a single dose or fractionated doses with daily or weekly whole-body exposure to ^{60}Co γ-rays or ^{235}U fission-spectrum neutrons. One of the observations from the study was an enhanced incidence of lung adenocarcinomas in mice exposed to protracted neutron doses, compared with those receiving the same total dose via a single-dose exposure in the $B6CF_1$ male mice (1). The purpose of these experiments was to identify multigene alterations related to the high incidence of lung adenocarcinomas from mice exposed to protracted neutron irradiation.

***mRb* and *p53* Deletions.** Past work has shown that the *mRb* gene was frequently deleted in both mouse and human lung adenocarcinomas (2,3). *mRb* is a recessive tumor suppressor gene that requires both alleles to be inactivated for tumor formation. *p53* is atypical among tumor suppressor genes in that one mutated allele will give cells heterozygous for that mutation a selective growth advantage and lead the cell to transformation (4).

K-*ras* Codon 12 Point Mutation. *ras* genes can be activated predominantly by point mutations and have been found to be activated by radiation (5). Mutation of *ras* gene stimulates cellular growth and transformation (6,7).

MATERIALS AND METHODS

One hundred twenty paraffin-embedded lung tissues were randomly selected and tested in this study. Eighty tissue samples from irradiated normal lungs or lung adenocarcinomas of mice exposed to 24 once-weekly γ-ray irradiation (24×1,γ), 60 once-weekly γ-ray irradiation (60×1,γ), 24 once-weekly neutron irradiation (24×1,n), and 60 once-weekly neutron irradiation (60×1,n); Forty tissue samples from nonirradiated controls (20 spontaneous lung adenocarcinomas and 20 sham-irradiated normal lungs).

Detection of *mRb* and *p53* deletions was carried out by Polymerase Chain Reaction (PCR) followed by Southern blot analyses. The method was adapted from previous experiments from the laboratory (2). K-*ras* codon 12 point mutation was detected by modifying the Enriched-PCR method (8). β_2-Microglobulin was used as an internal control for the PCR amplification.

The statistical analyses for the data were done by "Fisher's exact test" (2 × 2 contingency table), the critical value (α) was 0.05.

RESULTS AND DISCUSSION

Table 1 is the summary for *mRb* deletion from each irradiated and control group. A significantly higher frequency of *mRb* deletion was observed in lung adenocarcinomas of mice exposed to 60×1,γ irradiation (4 of 10; 40%) than those exposed to low-dose and low-dose-rate 24×1,γ irradiation (0 of 10, 0%; $P < 0.05$); however, the 60×1,γ deletion frequency was not significantly different from that in lung adenocarcinomas of mice that had single-dose γ-ray exposure (1 of 6l; 17%) with a similar total dose or from that in spontaneous mouse lung adenocarcinomas (5 of 20; 25%). No statistically significant differences of dose or dose-rate effects were observed in *mRb* deletion freqeuncies from normal lungs and lung adenocarcinomas of mice exposed to single or protracted doses of neutron radiation.

Twelve (20%) of 60 lung adenocarcinomas have shown *mRb* deletion. Among all of the normal lungs and lung adenocarcinomas that had deleted *mRb*, *mRb* fragments 3 (71%) and 5 (67%), the parts of the gene that encoded the pocket binding region of Rb protein to adenovirus E1A and SV40 T-antigen, were the most frequently deleted fragments (Table 3).

The detection of K-*ras* oncogene codon 12 point mutations was performed in the same set of 120 paraffin-embedded lung tissues (Table 2). The study revealed the increased effectiveness of high-LET (linear-energy-transfer) neutron radiation over low-LET γ-ray irradiation for the induction of K-*ras* codon 12 point mutations in the lungs of $B6CF_1$ mice. A significant increase in K-*ras* codon 12 point mutations was observed in 24×1,n irradiated normal lungs (10 of 10; 100%) when

compared with normal lungs that were sham-irradiated controls (5 of 10; 50%). Lung adenocarcinomas of mice receiving 24×1,n (10 of 10; 100%) also had a significantly higher frequency of K-*ras* codon 12 point mutations than did the lung adenocarcinomas of mice receiving 24x1,γ or 60x1,γ irradiation (5 of 10; 50%); however, the 24×1,n mutation frequency in lung adenocarcinomas was not significantly different from that for spontaneous mouse lung adenocarcinomas (15 of 20; 75%). The validity of enriched-PCR for detecting K-*ras* codon 12 points mutation was confirmed by sequencing two of the mutants, which showed a G to T or A transervation on codon 12 or 13 or both (data not shown).

The *p53* study was carried out by using the normal lungs and lung adenocarcinomas from mice that initially were found to have a deleted *mRb* gene (Table 3). Exons 1, 4, 5, 6 and 9 of *p53* were chosen to be examined. The data from these experiments demonstrated a high frequency of *p53* deletion in the selected lung tissues (30 of 31; 97%), all lung adenocarcinomas had *p53* deletion (15 of 15; 100%). Exon 4 (83%) and exon 5 (90%), parts of the gene that encode the core domain of p53 protein (exon 5-8), were the most frequently deleted exons. Complete *p53* deletions were found in a higher percentage of the mice exposed to 60×1,n (5 of 8; 63%) than the mice exposed to 60×1,γ (2 of 7; 29%).

Taken together, *p53* and K-*ras* were two of the contributing factors responsible for the increased incidence of lung adenocarcinomas in $B6CF_1$ male mice exposed to protracted neutron irradiation.

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TABLE 3. *mRb/p53/K-ras* Gene Alterations in Spontaneous and Irradiated Lung Adenocarcinomas from $B6CF_1$ Mice

Paraffin blocks	Radiation/ reatmen	*Fragments of mRb* deleted	Exons of *p53* deleted	K-*ras* (codon 12)
BCF1 (+ control)	0	None	None	None[a]
S0835(N)[b]	0	1,2,3,4,6	1,4,5,6,9	m[c]
C2462(N)	0	None	1,4,6	None
S0644(N)	0	None	4,5	m
S2379(N)	0	None	1,4,5	None
C1343(T)[d]	24×1,γ	None	1,4,5,9	m
S0473(N)	60×1,γ	1,2,3,4,5,6	1,5	m
S0897 (N)	60×1,γ	2,3,4,5,6	4,5,6	None
S1840 (N)	60×1,γ	3,5,6	None	None
S1134 (T)	60×1,γ	2,3	1,5,6	m
S1406 (T)	60×1,γ	1,2,3,4,5,6	1,4,5,6,9	m
S1458 (T)	60×1,γ	1,2,3,4,5,6	1,4,5,6,9	m
S2109 (T)	60×1,γ	3	1,4,5,9	None
C3576 (T)	Spontaneous	3	5	None
C4492 (T)	Spontaneous	5	1,4,5,6	m
C3495 (T)	Spontaneous	3,4,5	1,4,5,6,9	m
S0762 (T)	Spontaneous	1,2,3,4,5,6	1,4,5,6,9	m
S1591 (T)	Spontaneous	None	5	m
S2123 (T)	Spontaneous	None	4,5,6	m
S1229 (T)	Spontaneous	5	N/A[e]	None
C0591 (N)	24×1,n	None	1,4,5,9	m
C3176 (N)	24×1,n	1,3	1,4,5,6	m
C4260 (N)	24×1,n	1	4,6	m
C1515 (T)	24×1,n	None	4,6	m
S0239 (N)	60×1,n	2,4,5	4,5	m
S0584 (N)	60×1,n	1,2,3,4,5,6	1,4,5,6,9	m
S2051 (N)	60×1,n	None	5	m
S1166 (N)	60×1,n	3,4,5	1,4,5,6,9	m
S0550 (T)	60×1,n	2,5	1,4,5,6,9	m
S0263 (T)	60×1,n	1,2,3,4,5	1,4,5,6,9	m
S0945 (T)	60×1,n	1,2	1,4,5,6,9	m
S2363 (N)	60×1,n	None	4,5	m

[a] None = No K-*ras* codon 12 point mutation.
[b] N = Normal mouse lung.
[c] m = Point mutation of K-*ras* codon 12.
[d] T = Mouse lung tumor.
[e] N/A = not available.

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TABLE 1. Incidence of Partial or Total *mRb* Deletion in Mouse Lung Adenocarcinoma

Treatment	Normal lung (sham-irradiated)	Spontaneous lung tumor	Normal lung (n)	Lung tumor (n)	Normal lung (γ)	Lung tumor (γ)
Single dose[a]	0/6 (0%)	6/18 (33%)	0/6 (0%)	0/6 (0%)	0/6 (0%)	1/6 (17%)
24 × 1	0/10 (0%)	5/20 (25%)	2/10 (20%)	0/10 (0%)	0/10 (0%)	0/10 (0%)
60 × 1	1/10 (10%)	–	3/10 (30%)	3/10 (30%)	3/10 (30%)	4/10[b] (40%)

[a] Data from Reference 2.
[b] Significantly different from lung adenocarcinomas of mice receiving 24×1,γ irradiation ($P = 0.043 < \alpha = 0.05$; Fisher's exact test); but not significantly different from spontaneous lung adenocarcinomas ($P = 0.431 > \alpha = 0.05$) or lung adenocarcinomas of mice receiving 1×1,γ (P = 0.588).

TABLE 2. Incidences of K-*ras* Gene Codon 12 Point Mutations in Spontaneous Mouse Lung Adenocarcinomas and Lung Adenocarcinomas from Radiation-exposed $B6CF_1$ Mice

Treatment (total dose)	Normal lung (sham-irradiated)	Spontaneous lung adenocarcinoma	Normal lung (neutron)	Lung tumor (neutron)	Normal lung (γ-ray)	Lung tumor (γ-ray)
24x1 (n = 60 cGy; γ = 417cGy)	5/10 (50%)	15/20 (75%)	10/10[#] (100%)	10/10[@] (100%)	1/10 (10%)	5/10 (50%)
60x1 (n = 40 cGy; γ = 600cGy)	7/10 (70%)	-	9/10 (90%)	8/10 (80%)	3/10 (30%)	5/10 (50%)

[a] Significantly different from normal lungs of mice receiving 24x1 sham-irradiation ($P = 0.016$).
[b] Significantly different from lung adenocarcinomas of mice receiving 24x1,γ or 60 x1,γ irradiation ($P = 0.016$); but not significantly different from mouse spontaneous lung adenocarcinomas ($P = 0.140$) and not significantly different from adenocarcinomas of mice receiving 60x1,n irradiation ($P = 0.474$).

DISCLAIMER