Short Communication

p53 and K-ras in Radon-associated Lung Adenocarcinoma

Josh W. McDonald, Jack A. Taylor,[1] Mary A. Watson, Geno Saccomanno, and Theodora R. Devereux

Laboratory of Molecular Carcinogenesis [J. W. M., M. A. W., T. R. D.] and Epidemiology Branch [J. A. T.], National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709, and St. Mary's Hospital, Grand Junction, Colorado [G. S.]

Abstract

Mutations in the *p53* tumor suppressor gene and the K-*ras* proto-oncogene are common genetic defects in lung cancer. Analysis of the patterns of damage in these genes may provide important insights into the mechanisms by which environmental mutagens initiate cancer. Previously, our laboratory found that a rare p53 codon 249 mutation (AGG^{ARG} to ATG^{MET} transversion) was present in 31% of a series of 52 large and squamous cell lung cancers from uranium miners, suggesting that this mutation might be a marker for radon exposure. In the current study, we analyzed 23 lung adenocarcinomas from the same cohort of highly exposed uranium miners. These tumors failed to show the codon 249 transversion, but 9 (39%) of 23 contained 1 or more mutations within hotspots in the K-*ras* gene. The results suggest that there is histological tissue-type specificity for the codon 249 mutation; although this mutation was common in squamous and large cell tumors from very highly exposed uranium miners, it is rare in adenocarcinomas from the same cohort of miners.

Introduction

Radon-222 gas is a naturally occurring decay product of radium-226, which is a member of the decay series of uranium-238. Radon gas accumulates in mines and gives rise to progeny that emit α-particles, making it the primary source of radiation exposure in uranium miners. α-Particles are known to cause DNA damage, primarily large deletions, but are also known to produce point mutations (1). Uranium miners have an increased risk of lung cancer, and this risk is further enhanced in miners who smoke (2). Radon exposure in homes may also be a significant cause of lung cancer in the general population, although recent studies have had conflicting findings (3).

Mutations in the tumor suppressor gene *p53* are the most common known type of genetic defect in all types of lung cancer. Adenocarcinomas of the lung also commonly contain mutations in the proto-oncogene K-*ras*, but K-*ras* mutations are reported to be absent in lung tumors from uranium miners (4). Analysis of the patterns of molecular damage in the *p53* and K-*ras* genes is important because it provides insight into the mechanisms by which environmental agents such as radon induce lung tumors.

Previously, our laboratory analyzed *p53* defects in large and squamous cell lung carcinomas from 52 uranium miners (Colorado Plateau, CO) and found that a significant proportion (31%) of these tumors had an AGG^{ARG} to ATG^{MET} transversion at codon 249 of *p53* (5). Although other codon 249 mutations are common in certain malignancies, the transversion at the second position is rare, having been reported in only 6 of 2556 (0.2%) *p53* mutations from a database of human tumors (6), after excluding the tumors from our previous report (5). Our previous data suggested that the codon 249 mutation may be a marker for radon-induced lung cancer and raised the question as to whether the same mutation was present in malignancies of other histological types with this same exposure.

In the current study, we analyzed 23 lung adenocarcinomas from uranium miners and 6 such tumors from nonminers for mutations in key hotspots: codon 249 of *p53* and codons 12, 13, and 61 of K-*ras*.

Materials and Methods

Adenocarcinomas from 23 uranium miners of the Colorado Plateau were obtained as formalin-fixed, paraffin-embedded specimens (22 cases) or as fresh-frozen tissue (1 case) from St. Mary's Hospital (Grand Junction, CO). Most (20 samples) represented tissue from primary lung tumors; the remaining 3 cases represented metastatic deposits from patients with a known primary lung adenocarcinoma. Although all miners had long-term exposure (Table 1), for 3 miners the occupational exposure could not be quantified; the remaining 20 miners had a mean exposure of 1270 working level months (1.3×10^5 MeV potential α-energy/liter air $\times$ working month exposure). Six fixed adenocarcinomas from nonminers were also obtained from St. Mary's Hospital as a nonradon-exposed comparison group. All uranium miners and three of the six nonminers had smoked cigarettes; the mean usage history for the miners was 62 pack-years. Before genetic analysis, all tissue samples were histologically examined to verify tumor histology and grade.

DNA was isolated from tissue sections by using overnight proteinase K digestion at 37°C, followed by phenol-chloroform extraction and ethanol precipitation. Exon 7 of *p53* and exons 1 and 2 of K-*ras* were individually amplified by sequential PCRs, with nested outer and inner primers flanking the codons of interest. Amplification was performed on a Perkin Elmer Cetus Thermal Cycler. For exon 7 of *p53*, the PCR conditions were: 30 cycles of 1 min at 94°C, 1 min at 62°C, and 30 s with 1 s/cycle extension at 72°C for the outer reaction, followed by 35 cycles of 1 min at 94°C, 1 min at 62°C, and 1 min at 72°C for the inner reaction. For exons 1 and 2 of K-ras, the PCR conditions were: 30 cycles of 1 min at 94°C, 1 min at 50°C, and 30 s with 1 s/cycle extension at 72°C for the outer reaction, followed by 35 cycles of 1 min at 94°C, 1 min at 50°C, and 1 min at 72°C for the inner reaction. Codon 249 of the *p53* gene was examined by using the previously described (5) PCR-based RFLP analysis with *Bsa*BI, an enzyme which cleaves only the

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[1] To whom requests for reprints should be addressed, at Epidemiology Branch, National Institute of Environmental Health Sciences, P. O. Box 12233, Research Triangle Park, NC 27709.

Table 1 K-ras mutations in lung adenocarcinomas from uranium miners

Miner	Grade	Codon	Mutation	Amino Acid substitution	Smoking pack-yr	Radon WLM
1	1[a]	12	GGT → GAT	gly → asp	27	4403
2	1	12	GGT → GAT	gly → asp	8	829
3	2[b]	12	GGT → GAA	gly → glu	84	1000
		61	CAA → CGA	gln → arg		
4	3[c]	13	GGC → GAC	gly → asp	108	1129
		61	CAA → CGA	gln → arg		
5	1	12	GGT → GTT	gly → val	66	824
6	2	12	GGT → TGT	gly → cys	78	NA[d]
7	3	13	GGC → TGC	gly → cys	102	69
8	2	12	GGT → TGT	gly → cys	144	553
9	2	12	GGT → TGT	gly → cys	60	845

[a] 1, well differentiated.
[b] 2, moderately differentiated.
[c] 3, poorly differentiated.
[d] NA, not available.

mutant ATG sequence. This assay utilized a 2-h incubation of amplified DNA with 1 unit *Bsa*BI (New England Biolabs, Beverly, MA) at 60 C, followed by denaturation and electrophoresis on an 8% polyacrylamide gel. On the basis of our previous work (5), this assay was as sensitive as direct sequencing for detecting the codon 249 mutants. As a test of the PCR-RFLP technique, we included three squamous and large cell lung tumors known to contain the codon 249 mutation; these consistently tested positive in our assay. As additional verification, ten adenocarcinomas from miners shown to be negative by PCR-RFLP analysis were sequenced by the Sanger dideoxy chain termination method and shown to contain only the wild-type codon 249 sequence.

All samples were also assayed for mutations in exon 1 of K-*ras* at codons 12 and 13 by direct sequencing; all positive samples also had abnormal band migration by single-strand conformation polymorphism analysis. For exon 2 of K-*ras*, amplified DNA samples were screened for mutations using single-strand conformational polymorphism by using two different gel migration conditions [8% polyacrylamide and 10% glycerol and 0.5× MDE (AT Biochem, Malvern, PA) and both were run at 6–8 W at room temperature]. Samples with abnormal migration were sequenced, again by using the dideoxy chain termination method.

Results

In contrast to large and squamous cell lung cancers from Colorado uranium miners, where 16 of 52 (31%) had a *p53* codon 249 AGG^{ARG} to ATG^{MET} transversion (5), 0 of 23 adenocarcinomas from the same cohort of uranium miners, and 0 of 6 adenocarcinomas from nonminers had this mutation.

Eleven missense mutations in codons 12, 13, or 61 of K-*ras* were detected in adenocarcinomas from 9 of 23 (39%) uranium miners (Table 1). Seven of 11 (64%) mutations were in codon 12, 2 (18%) in codon 13, and 2 (18%) in codon 61. The 2 adenocarcinomas with codon 61 mutations also had defects at one of the other codons. Three (27%) of 11 of the mutations were G to A transitions, and 5 (45%) were G to T transversions, all of which were found in codon 12 or 13. One tumor had a GGT^{GLY} to GAA^{GLU} double mutant within codon 12 (Table 1); our method could not distinguish between a double mutation of the same allele and single point mutations of separate alleles. The remaining two mutations (18%) were found in codon 61 and were A to G transitions. No K-*ras* mutations were found in the comparison group of the six nonminers.

Discussion

The lack of detectable *p53* codon 249 transversions in our series of adenocarcinomas is an unexpected result, given the frequency of this mutation in our previous report of squamous and large cell tumors from the same cohort of miners (5). Lo *et al.* (7) recently reported that, in their series of lung carcinomas associated with domestic radon exposure, 0 of 10 squamous cell cancers, 0 of 8 adenocarcinomas, and 0 of 3 undifferentiated tumors had the 249 mutation. However, these individuals had exposure levels generally between 2 and 3 orders of magnitude lower than the occupational exposures experienced in our cohort of miners.

Our failure to find codon 249 mutations in adenocarcinomas from uranium miners cannot be explained by lower dose levels than in our previous study of squamous and large cell lung tumors: the average radon exposure of the former group was 1270 working level months, similar to the average of 1382 working level month exposure for the patients in the latter group. Nor can the lack of 249 mutations in adenocarcinomas be attributed to assay insensitivity because DNAs extracted from known positive squamous and large cell tumors continued to be positive when assayed in the *Bsa*BI RFLP procedure.

It is possible that the presence of the codon 249 mutation in radon-associated squamous and large cell tumors but not in adenocarcinomas reflects a cell-type specific clonal selection process. Cells acquiring the codon 249 mutation might preferentially develop into squamous and large cell tumors. Alternatively, it is possible that although these adenocarcinomas occurred in uranium miners, radon was not responsible for their pathogenesis. Of lung tumor types, adenocarcinoma has the weakest association with radiation exposure in both uranium miners and atomic bomb survivors (2). In addition, *p53* mutations are less prevalent in adenocarcinoma (33%) than in other common histological types of lung cancer (60–70%; Ref. 8) so damage to the *p53* gene may represent a less common pathway for cancer development in this tumor type. Because we have restricted our analysis of *p53* to a single mutational hotspot, we have not excluded the possibility that radon may have caused damage at other sites in the *p53* gene.

The finding of 9 (39%) of 23 adenocarcinomas with K-*ras* mutations in our series is similar to the 22–46% frequency reported in smoking-associated adenocarcinoma (9, 10). Despite this relatively high mutational frequency, a recent series by Vahakangas *et al.* (4) reported that K-*ras* mutations were absent in 19 lung cancers from uranium miners. However, this series (4) included only 2 adenocarcinomas; the remaining 17 were other histological types in which K-*ras* has been found to be infrequently mutated (9).

The pattern of K-*ras* bp changes in our series of lung tumors from uranium miners is largely similar to those reported in smoking-associated adenocarcinomas. In particular, G to T transversions, which accounted for 5 of the 11 mutations we found, are an especially common type associated with tobacco smoke exposure in studies of *p53* and K-*ras* (8, 11). The hypothesis that carcinogens in tobacco smoke were responsible for the K-*ras* mutations in this series is supported by the higher mean usage history (75 pack-years) in the 9 miners with mutations, compared to the figure (54 pack-years) for the 14 miners without mutations, although this difference did not achieve statistical significance ($P = 0.17$). Because G to T

transversions have been observed in the setting of *in vitro* mutagenesis by α-particle radiation (1) and were the most common mutation in our previous study of large and squamous cell tumors in uranium miners (5), we cannot exclude the possibility that radon exposure contributed to one or more of these mutational events.

In summary, our data are consistent with the finding of other studies that point mutations in key oncogenes and tumor suppressor genes may show distinct differences in frequency across various histological types of cancer. In particular, the *p53* codon 249 AGG^{ARG} to ATG^{MET} transversion we earlier reported for radon-associated squamous and large cell lung tumors (5) appears unlikely to be important in the genesis of adenocarcinomas in uranium miners. There may be other as yet unexplored genetic defects present in these adenocarcinomas that would reflect radon as an etiological factor. Conceivably, cells acquiring the codon 249 defect may preferentially develop into large and squamous cell tumors rather than into adenocarcinomas. Alternatively, given the weaker association between radon exposure and adenocarcinoma of the lung (2), it is possible that these tumors were caused by other factors such as smoking.

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