Differential Dose Responses of Pulmonary Tumor Types in the Rat after Inhalation of Plutonium Dioxide Aerosols

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Rats/239PuO2/Alpha dose/Lung tumors/Phenotypes

Dose responses were compared among primary lung tumors and their histological types induced by a single inhalation exposure of female Wistar strain rats to submicron-size and polydispersed aerosols of plutonium dioxide (239PuO2). While the primary lung tumors were found only in 2.3% of the unexposed control animals, the frequency of all the primary lung tumors in the exposed animals was 44% at the mean lung dose of 0.71 Gy, and increased sharply at the doses of 1.5 Gy or more, reaching the maximum of 97% at 5.4 Gy, and the dose responses around at 1.0 Gy were different between benign and malignant lung tumors. Almost all the pulmonary tumors in the exposed animals were classified into epithelial types such as adenomas, adenocarcinomas, adenosquamous carcinomas, and squamous cell carcinomas. The dose responses were different between these tumor types as shown by the peak incidence of adenomas at 0.71 Gy, adenocarcinomas at 2.9 Gy, adenosquamous and squamous cell carcinomas at 5.4–8.5 Gy, respectively. As the magnitudes of neoplastic lesions in pulmonary carcinomas were expressed by histological scores, metaplasias and adenomatous lesions most frequently appeared at doses of 1.5 Gy, while the appearance and increase of carcinomatous lesions differed in the dose ranges as shown by the peak incidence of adenocarcinomatous lesions at 2.9 Gy, and adenosquamous or squamous lesions at 5.4–6.6 Gy. These results indicate a differential dose response of pulmonary carcinogenesis in which metaplasias and benign adenomas were induced at lower doses (< 1.0 Gy), whereas malignant carcinomas were induced at relatively higher doses (> 1.5 Gy). Together with the increase of carcinomatous lesions at higher doses, the intranuclear p53 protein accumulation was detectable, but only in a few percentages of malignant carcinomas.
INTRODUCTION

The human dosimetric model for radiation protection against inhalation exposures to air-borne radioactive aerosols has been described as limits for intakes of radionuclides by workers, in ICRP 30\(^1\), which has recently been revised in ICRP 66\(^2\) as a new human respiratory tract model with anatomical, morphological, and physiological parameters to permit the calculation of the deposition and dose for inhaled particles in different human subjects. The biological effects of inhaled radionuclides on the target organs at risk following the inhalation exposures have been summarized in ICRP 31\(^3\), and the linear dose response with no threshold in low dose levels’ exposures to various sources of ionizing radiation has been proposed for the potentially carcinogenic effects of some inhaled radionuclides\(^4\). While it remains unknown whether the inhalation exposures to the air-borne plutonium, both \(^{238}\text{Pu}\) and \(^{239}\text{Pu}\), might result in the increased lung cancer risks in humans, the epidemiological investigations on occupational exposures of nuclear workers to plutonium and other radiation sources, however, implies no significant evidence for the increase of lung cancer mortality as compared to that of the age-matched populations\(^5\),\(^6\). Only the animal studies can therefore provide an experimental evidence for the risk estimation of inhaled plutonium. The estimated lifetime pulmonary cancer risks of inhaled plutonium aerosols with chemically different forms in beagle dogs\(^7\),\(^8\) are derived from an approximation of the quadratic dose response curves rather than a pure linear one with no threshold, while the experiments using both Wistar and Fischer 344 strain rats\(^9\),\(^10\),\(^11\),\(^12\) after inhalation exposures to high-fired plutonium dioxide aerosols, however, have indicated a “practical” threshold at low doses around 1 Gy for the dose response of malignant lung carcinomas as compared to much lower frequencies of spontaneous lung tumors. Although the reasons and underlying mechanisms for such a threshold-like dose response of pulmonary malignancies remain unclear, the distribution of aerosol particles in the respiratory tract and alpha doses or dose rates to the lining epithelium might result in differential dose responses of neoplastic lesions. The relationships between dose and tumor malignancies by inhalation of alpha particles-emitted radionuclides are not, however, fully elucidated.

We previously reported a high incidence of malignant lung carcinomas at the lung doses of 4–12 Gy from the life-span animal study on pulmonary carcinogenesis in female Wistar strain rats exposed to submicron-size and polydispersed \(^{239}\text{PuO}_2\) aerosols\(^13\). The present paper summarizes the current results on differential dose responses of pulmonary carcinomas found at the doses of 0.71–10 Gy, in a special reference to the malignancies and histopathological phenotypes of tumors, as well as the frequency of the intranuclear p53 protein accumulation as an indicator for malignant phenotypes resulted from the mutation of the tumor-suppressor gene\(^14\).

MATERIALS AND METHODS

Experimental animals, inhalation exposures and care

Because of slower increase of the body weight and easier handling than the males, and no.
significant differences in the lung tumor induction noted between both sexes\textsuperscript{11,12}, we used 8-week-old female Wistar strain (W/M) rats, which were purchased from our animal breeding facility, fed on a commercial diet with water \textit{ad libitum}, and kept under a barrier-filtered air before and after the experiments. Following the previous training, 20 healthy rats (80- to 100-day-old) were placed each into a plastic hand-made holder for nose-only exposures and exposed once for a maximum of 60 min to high-fired $^{239}\text{PuO}_2$ aerosols in a multiport inhalation chamber device as described below. All the age-matched, exposed and unexposed control animals were housed five per a polycarbonate cage and kept in a closed hood rack placed in the animal rooms during their lifetimes until the death. The animal rooms were maintained on a 12 hr-daylight cycle with an air temperature of 23 ± 1.0°C and a humidity of 55 ± 5.0%. Animal care consisted of weekly change of clean cages and daily checking of the animals' conditions for survival and immediate pathological examinations of dead animals.

\textit{Generation and assessment of aerosols}

As previously described\textsuperscript{13}, the plutonium hydroxide solution chemically converted from the nitrate solution was nebulized in a compressed air-driven nebulizer, passed through a tube heated at 300°C to dry the droplets in air, and then conducted into a high temperature furnace heated at 1150°C. An air flow containing high-fired $^{239}\text{PuO}_2$ aerosol particles was introduced through a negative pressure by an exhaust air-pump into a multiport inhalation chamber device. Aerosol samples were collected simultaneously on a 10-stage cascade impactor plate to determine the particle size distribution. The activity median aerodynamic diameter (AMAD) was ranged from 0.3 to 0.5 μm with a geometric standard deviation of 1.8–2.1 in all the experiments, indicating the generation of submicron-size and polydispersed aerosol particles.

\textit{Determination and calculation of lung doses}

The initial lung deposition (ILD) was determined individually in the exposed rats by the whole body counting of the low energy LX-ray of 17 keV emitted from $^{239}\text{Pu}$ deposited in the lung, and the cumulative alpha dose absorbed in the entire lung tissue was calculated at the time of death, based on the time-integral of the ILD, retention function, and lung weights as previously described\textsuperscript{13}. In the following description, the dose refers to the mean of cumulative lung dose in each group, unless otherwise mentioned.

\textit{Pathological examinations}

The postmortem pathological examinations were available on total 440 (130 control and 310 exposed) rats after the lifetimes. At autopsy, the gross lesions were carefully examined, in particular as to the distribution of neoplastic foci in each lobe of the lung, trachea, bronchi, and tracheobronchial lymph nodes. These tissue samples and the other main organs were fixed in 10% phosphate-buffered formalin, cut into small pieces, processed with graded ethanol and xylene in an automatic tissue processor, and then were embedded in paraffin. Sections, 5 to 6 μm-thick, were prepared and stained with hematoxylin and eosin (HE) for the light microscopic examinations unless otherwise mentioned.

Differential diagnosis of the primary lung tumors from metastatic ones and between the
histological types was based on the WHO criteria\(^{15}\). All the primary lung tumors except for 2 non-epithelial types (fibrosarcomas or hemangiosarcomas) were classified into epithelial types such as ductal or papillary adenomas, ductal, papillary, or solid adenocarcinomas, solid and non-keratinized adenosquamous carcinomas, and keratinized and epidermoid squamous cell carcinomas, as previously described\(^{13}\). The magnitudes of metaplastic or neoplastic lesions in each histological specimen from these epithelial types of tumors, were further evaluated under a light microscopic field by the following histological score criteria: 0 point as negatively observed, 0.5 point as slightly observed in 1–2 areas, 1.0 point as mildly observed in 3–4 areas, and 2.0 point as severely observed in 5 areas or more. The sum of those scores given for each neoplastic lesion was compared between the groups of animals received various doses.

The immunohistochemical staining of the intranuclear p53 protein was applied on all the histological specimen from epithelial types of tumors by a modified method as described\(^{14}\). Briefly, deparaffinized sections on the silanized slide immersed into an antigen retrieval solution (DAKO Corp., Carpinteria, CA) were heated to 90–95°C for 30 min in a microwave oven to unmask antigens, and rinsed with phosphate-buffered saline (PBS). After the blocking of endogenous peroxidase activity with 0.3% H\(_2\)O\(_2\) in methanol and of non-specific antibody binding with normal goat serum, either polyclonal rabbit serum (CM1) or monoclonal mouse IgG (DO-7), specific to both mutant and wild type human p53 protein, from Medac Inc., Germany, was applied at optimal concentration of 1/100–1/250 on pretreated sections. After the incubation at 4°C overnight, the sections were washed with PBS, then the biotinylated secondary antibody, anti-rabbit or anti-mouse IgG, and avidin-biotin-peroxidase complex (ABC) were reacted respectively with sections by using a commercial kit (Vectastain\textsuperscript{®} ABC kit; Vector Laboratories, Inc., Burlingame, CA), and finally the peroxidase activity was visualized by using 3, 3’-diaminobenzidine (DAB) as a substrate. The positivity for the intranuclear accumulation of p53 protein detected under a light microscopic field was evaluated by the following criteria; (−) as negatively detectable in all nuclei, (±) slightly detectable in 10% or less, (+) as mildly positive in 20–40%, and (++) as severely positive in 50% or more of all nuclei of tumor cells, respectively.

Statistics

The survival period and lung tumor incidences were compared for significant differences between the control and exposed groups of animals by Student’s \(t\)-test.

RESULTS

Lung dose, survival, and primary lung tumors after inhalation exposures

As shown in Table 1, total 310 exposed rats were divided into 7 groups based on the mean values of initial lung deposition and cumulative lung dose ranging from 0.71 to 8.5 Gy. As compared to the group of unexposed control animals, the mean survival period of the lowest dose (0.71 Gy) group significantly (\(p < 0.01\)) increased, while those of the groups received doses more than 1.5 Gy were significantly (\(p < 0.01\) or 0.001) reduced, and dropped to almost 60% of the control value at 8.5 Gy. The crude incidence of the primary lung tumors was 44% at 0.71 Gy (p
<0.001), increased sharply at the doses of 1.5-4.7 Gy, and reached the maximum of about 97% at 5.4 Gy (p < 0.001) as compared to the control group which had only 3 primary tumor-bearing animals (2.3%) among 130 animals. Among these tumors, benign ones showing a localized growth of adenomatous foci were most frequently observed at the doses less than 1 Gy and decreased at higher doses over 1.5 Gy, whereas, malignant ones showing a widely spread and disseminated growth of carcinomatous or sarcomatous foci were much less at the doses less than 1 Gy, but sharply increased up to 35-80% at the doses of 1.5-4.7 Gy, and reached the maximum of 90% at 6.6 Gy as shown in Fig. 1.

The histological types of these primary lung tumors observed in the control and each of the dose groups were summarized in Table 2. While the control had only 2 adenomas (1.5 %), the crude incidence of benign adenomas was the highest (35%) at 0.71 Gy, but decreased at the doses of 1.5 Gy or more, then reaching the bottom at 8.5 Gy. The malignant carcinomas including adenocarcinomas, adenosquamous carcinomas, and squamous cell carcinomas, were much less in the control (0.8%; squamous cell carcinoma) and in the lowest dose group (9.3%; adenocarcinomas), but their crude incidences were increased sharply at higher doses of 1.5 Gy or more. The appearance and the increase of each carcinoma type were, however, differed in the dose ranges; adenocarcinomas were appeared at 0.71 Gy, increased and reached the peak of 43% at 2.9 Gy, adenosquamous carcinomas were appeared at 1.5 Gy, increased and reached the peak of 50% at 8.5 Gy, squamous cell carcinomas were appeared at 2.9 Gy, increased and reached the peak of 13% at 5.4 Gy, respectively. Other tumor types such as fibrosarcomas and hemangiosarcomas were found only in 2 animals (3.2%) at higher doses of 6.6 Gy or more.

Dose-related appearance and increase of neoplastic lesions in pulmonary carcinomas

Almost all the primary lung tumors were epithelial types, and their histological types were expressed as benign or malignant phenotypes in a dose-dependent manner as above described. Actually, not only metaplasia of the alveolar lining epithelium but also variable stages of neo-

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>No. of Animals</th>
<th>Initial Lung Deposition (Bq)</th>
<th>Lung Dose (Gy)</th>
<th>Survival Period (Day)</th>
<th>Primary Lung Tumors (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>130</td>
<td>0</td>
<td>0</td>
<td>790 ± 144</td>
<td>34 (2.3)</td>
</tr>
<tr>
<td>Pu 1</td>
<td>43</td>
<td>97 ± 27</td>
<td>0.71 ± 0.19</td>
<td>871 ± 105*</td>
<td>19 (44.2)**</td>
</tr>
<tr>
<td>Pu 2</td>
<td>75</td>
<td>225 ± 48</td>
<td>1.52 ± 0.28</td>
<td>712 ± 162*</td>
<td>45 (60.0)**</td>
</tr>
<tr>
<td>Pu 3</td>
<td>60</td>
<td>461 ± 118</td>
<td>2.88 ± 0.51</td>
<td>631 ± 158**</td>
<td>46 (76.7)**</td>
</tr>
<tr>
<td>Pu 4</td>
<td>40</td>
<td>787 ± 79</td>
<td>4.67 ± 0.24</td>
<td>675 ± 98**</td>
<td>37 (92.5)**</td>
</tr>
<tr>
<td>Pu 5</td>
<td>31</td>
<td>948 ± 76</td>
<td>5.43 ± 0.29</td>
<td>622 ± 105**</td>
<td>30 (96.7)**</td>
</tr>
<tr>
<td>Pu 6</td>
<td>31</td>
<td>1147 ± 114</td>
<td>6.61 ± 0.28</td>
<td>550 ± 82**</td>
<td>29 (93.5)**</td>
</tr>
<tr>
<td>Pu 7</td>
<td>30</td>
<td>1672 ± 261</td>
<td>8.52 ± 0.67</td>
<td>458 ± 95**</td>
<td>27 (90.0)**</td>
</tr>
</tbody>
</table>

* Mean ± SD of the initially deposited activity per animal in each group.
** Mean ± SD of the cumulative lung dose per animal in each group.
*** Mean ± SD of the survival period per animal in each group after the time of inhalation exposures.
**** The numbers (% crude incidences) of animals with primary lung tumors in each group.
Asterisks indicate a significant difference as compared to the controls (* p<0.01 or ** p<0.001).
plastic lesions were, however, observed in the same tissue specimen from an exposed rat under a light microscopic field. Further detailed analyses on the magnitudes of such metaplastic and neoplastic lesions in pulmonary carcinomas were performed by evaluating their histological scores in relation to the mean lung doses. As shown in Fig. 2, the sum of histological scores for both metaplasias and adenomatous lesions were the highest at 1.5 Gy and decreased at the doses of 2.9 Gy or more, whereas the scores for adenocarcinomatous lesions sharply increased at the doses of 1.5 Gy or more, whereas the scores for adenocarcinomatous lesions sharply increased at the doses of

### Table 2. Dose and histological types of primary lung tumors after inhalation of $^{239}$PuO$_2$ aerosol

<table>
<thead>
<tr>
<th>Mean Dose(Gy)</th>
<th>No. of Animals</th>
<th>No. of Lung Tumors$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Adenoma</td>
</tr>
<tr>
<td>0</td>
<td>130</td>
<td>2 (1.5)</td>
</tr>
<tr>
<td>0.71</td>
<td>43</td>
<td>15 (34.9)</td>
</tr>
<tr>
<td>1.52</td>
<td>75</td>
<td>19 (25.3)</td>
</tr>
<tr>
<td>2.88</td>
<td>60</td>
<td>13 (21.7)</td>
</tr>
<tr>
<td>4.67</td>
<td>40</td>
<td>5 (12.5)</td>
</tr>
<tr>
<td>5.43</td>
<td>31</td>
<td>3 (9.7)</td>
</tr>
<tr>
<td>6.61</td>
<td>31</td>
<td>1 (3.2)</td>
</tr>
<tr>
<td>8.52</td>
<td>30</td>
<td>0 (0.0)</td>
</tr>
</tbody>
</table>

$^a$ The numbers(% crude incidences) of animals with histological types of primary lung tumors in each dose group.

$^b$ Other types of tumors include fibrosarcomas and hemangiosarcomas.
1.5 Gy or more, reached the peak at 2.9 Gy, and decreased at the doses of 4.6 Gy or more. The scores for both adenosquamous and squamous lesions increased at the doses of 2.9-4.6 Gy, and reached the peak at 5.4-6.6 Gy, sustaining the plateaux at 8.5 Gy. Such dose-dependent appearance and increase of variable neoplastic lesions were almost correlated with the dose responses of histological types of lung tumors as shown in Table 2.

![Fig. 2. The relations of the neoplastic lesions in pulmonary carcinomas to the lung doses in rats after inhalation exposures to $^{239}$PuO$_2$ aerosols. The histological scores for each of the neoplastic lesions as illustrated in the legend are plotted against the mean lung dose (Gy).](image)

Table 3. Frequency of p53 protein expression in pulmonary carcinomas after inhalation of $^{239}$PuO$_2$ aerosol

<table>
<thead>
<tr>
<th>Histological Types of Pulmonary Carcinomas</th>
<th>No. Examined</th>
<th>No. of p53-Positive Carcinomas$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Negative (−) Slight (±) Mild (+) Severe (++)</td>
</tr>
<tr>
<td>Adenoma</td>
<td>56</td>
<td>56 0 0 0</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>97</td>
<td>85 11 1 0</td>
</tr>
<tr>
<td>Adenosquamous carcinoma</td>
<td>63</td>
<td>38 23 2 0</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>15</td>
<td>7 4 3 1</td>
</tr>
<tr>
<td>All types</td>
<td>231</td>
<td>186 38 6 1</td>
</tr>
</tbody>
</table>

$^a$ The numbers of pulmonary carcinomas showing intranuclear p53 protein with the variable grades detected by immunohistochemistry (see text).

1.5 Gy or more, reached the peak at 2.9 Gy, and decreased at the doses of 4.6 Gy or more. The scores for both adenosquamous and squamous lesions increased at the doses of 2.9-4.6 Gy, and reached the peak at 5.4-6.6 Gy, sustaining the plateaux at 8.5 Gy. Such dose-dependent appearance and increase of variable neoplastic lesions were almost correlated with the dose responses of histological types of lung tumors as shown in Table 2.

p53 expression in pulmonary carcinomas

The frequency of the intranuclear p53 protein accumulation detectable in each of the histological types of pulmonary carcinomas is shown in Table 3. All adenomas showed negative staining, and three types of carcinomas were mostly negative or slightly positive. The mildly or severely positive staining was found only in 7 carcinoma types among 231 tumors; 1 of 97 adenocarcinomas, 2 of 63 adenosquamous carcinomas, and 4 of 15 squamous cell carcinomas,
respectively. Although the frequency of p53-positive pulmonary carcinomas was therefore very low, both slightly and mildly or severely positive carcinomas were increased together with the increase of carcinomatous lesions at the doses of 2 Gy or more, whereas the number of p53-negative tumors were reduced at higher doses as the decrease of metaplastic and adenomatous lesions (Fig. 3).

Fig. 3. The relations of the neoplastic lesions and p53-positive carcinomas to the lung doses in rats after inhalation exposures to $^{239}$PuO$_2$ aerosols. The histological scores for either metaplastic and adenomatous lesions or carcinomatous lesions, together with the number of either p53-negative, slightly positive, or mildly and severely positive tumors as illustrated in the legend, are plotted against the upper limit of mean lung dose (Gy).

DISCUSSION

The present study showed a correlation between the survival reduction and increase of the primary lung tumors, especially malignant ones, in the $^{239}$PuO$_2$-exposed animals received the mean lung doses of 1.5 Gy or more. The mean survival period of the lowest dose (0.71 Gy) group of rats, however, significantly increased as compared to the controls, despite a significant increase of tumors which were, however, mostly benign adenomas found at later period after the inhalation exposures, suggesting that benign tumors are not the direct cause of death. These findings indicate a threshold-like dose range around at 1 Gy where the induction of benign or malignant lung tumors together with the survival reduction could differentially occur after inhalation exposures to $^{239}$PuO$_2$ aerosols. Although similar findings have been described elsewhere$^{10,11}$, the reasons and the background for a threshold-like response remain to be elucidated.

Among all the histological types of primary lung tumors found in the exposed animals, both adenomas and adenocarcinomas were the most frequent at the rate of about 66%, whereas either adenosquamous carcinomas or squamous cell carcinomas were respectively about 27% or 6.4%.
and non-epithelial sarcomas were only 0.8%. Such proportions of the tumor types are much differed from those described elsewhere in which adenomas and adenosquamous carcinomas were 10% or less, while squamous cell carcinomas were 30–50% among all the tumor types. Since variable aerosol sizes have been used in those studies, one of plausible reasons for the differences in the histological phenotypes might be attributed to the distribution of inhaled aerosol particles with different sizes which could affect the deposition sites in the upper or lower respiratory tract, resulting in different carcinogenic responses of the target lining epithelium variably sensitive to alpha particles. The dose responses were also differed among the histological types; the peak incidence of adenomas was found at the doses less than 1 Gy, whereas that of adenocarcinomas was at 2.9 Gy, adenosquamous carcinomas at 8.5 Gy, and squamous cell carcinomas at 5.4 Gy, respectively. These findings indicate a dose-dependent appearance and increase of the phenotypic expression of pulmonary carcinomas; higher doses could be required for the development of adenosquamous or squamous cell carcinomas, while adenomas or adenocarcinomas are inducible by relatively lower doses. Although the other investigations also implicated that squamous carcinomas more frequently increased in higher dose ranges than did adenocarcinomas as a result of the non-uniform dose distribution associated with plutonium particle aggregation either inside or outside of pulmonary regions, the susceptibility of the respiratory epithelium to carcinogenesis might be related to alpha doses and dose rates.

The evaluation on the magnitudes of neoplastic lesions by histological scores also indicated that both initial metaplasias and adenomatous lesions most frequently appeared at 1.5 Gy, while carcinomatous lesions increased in a dose-dependent manner as shown by the peak of adenocarcinomatous lesions at 2.9 Gy, and adenosquamous or squamous lesions at 5.4-6.6 Gy. Such a dose-dependent appearance of neoplastic lesions with variable stages suggests a successively phenotypic expression of the respiratory epithelium from an initially hyperplastic metaplasia to adenomatous, and then adenocarcinomatous or squamous metaplasias, reflecting a differential induction of variable histological types of pulmonary carcinomas as above described. The time course-dependent processes of neoplastic lesions are shown in both rats and mice sequentially sacrificed following the inhalation exposures to $^{239}$PuO$_2$; the respiratory epithelium consisted of either Type II alveolar cells or Clara cells, initially expressed hypertrophy or hyperplasia with the increased DNA synthesis from 30 to 90 days, and then metaplastic but pre-neoplastic changes in the middle stages around 180 days, followed by the onset of carcinomatous metaplasias in the later periods from 300 to 450 days, respectively after the exposures. It remains, however, to be elucidated whether such relatively early pathogenetic events in the pulmonary regions could be directly relevant to the long-term processes of the life-span carcinogenesis.

Dose-dependent and differential induction of pulmonary carcinomas with variable histological phenotypes as above described should be based on the cellular and molecular background such as an activation of protooncogenes and inactivation of tumor suppressor genes resulted from the mutations, leading to sequential appearance and expansion of pre-neoplastic cells, although there have scarcely been any of evidence on radiation-induced pulmonary carcinogenesis in experimental animals. For example, activation of K-ras oncogene as a result of the mutations could be related to an early pathogenetic event in human lung adenocarcinomas, while the mutations of a tumor suppressor gene, p53, are frequently detectable in human lung cancers from...
radon-exposed uranium miners with variable histopathological phenotypes\textsuperscript{21–24}. At least either or both of these genes are therefore considered as important candidates for mutations relevant to pulmonary carcinogenesis in humans. In contrast, experimental investigations on pulmonary carcinomas from the rats exposed to $^{239}$PuO$_2$ aerosols have shown that p53 mutations and intranuclear accumulation of mutant proteins were much less frequently detectable in carcinomatous lesions\textsuperscript{14}, whereas either K-ras activation or expression of growth factors were relatively well demonstrated\textsuperscript{25,26}. Our results on the p53 protein expression of pulmonary carcinomas showed that all the benign adenomas were negative, and most of the malignant carcinomas were negative or slightly positive, except that only a few percentages of mildly or severely positive carcinomas appeared at relatively higher doses together with the increase of carcinomatous lesions. Further detailed analyses should be required for the frequency of mutations and molecular sequences of the p53 gene locus extracted from these pulmonary carcinomas, to determine whether the frequency of p53 gene mutations may play a major role in the rat pulmonary tumors induced by $^{239}$PuO$_2$-exposures. Genetic alterations of p53, erbB2 and K-ras were recently reported to be rare in the canine pulmonary tumors induced by inhalation of $^{239}$PuO$_2$ aerosols\textsuperscript{27}. In addition, no alterations of K-ras and p53 genes are detected in X-ray-induced pulmonary tumors of the rat\textsuperscript{28}, whereas high frequencies of the p53 mutations and intranuclear protein accumulation are observed in the skin and bone tumors of mice after repeated beta particle-irradiation\textsuperscript{29}. These findings indicate the interspecies differences but also differences of radiation quality in genetic alterations related to radiation carcinogenesis, and further implicate the possibility that the molecular pathogenesis of radiation-induced pulmonary carcinomas in the rat might be differ from that of bone and lymphoid tumors induced in the mice by $^{239}$Pu-injection\textsuperscript{30}, or other radiation exposures.

In conclusion, the present results indicate a differential dose response between lung tumor types and neoplastic lesions induced by inhalation exposures of female Wistar rats to $^{239}$PuO$_2$ aerosols, while the pathogenetic processes and the molecular events including oncogenic mutations still remain unclear.

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