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Enhanced Progression from Lung Adenoma to Adenocarcinoma in Progeny by Prezygotic Testicular X-ray Exposure of Mice

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Abstract. : Testicles of nine-week-old male CBA/J mice were X-ray irradiated (1 Gy or 2 Gy) and were mated one week later with untreated virgin 12-week-old females of the same strain. The 1-Gy offspring (88 males and 62 females), 2-Gy offspring (100 males and 93 females) and additional offspring (83 males and 84 females) were treated once subcutaneously with 0.1 mg/g body weight of urethane at 6 weeks of age. These three groups of offspring showed similar incidences of lung tumors in both sexes. Depending on the doses of paternal X-ray irradiation, increasing incidences of adenocarcinoma were observed in the male 1-Gy and 2-Gy offspring groups.An increased multiplicity of lung carcinomas was observed in the male 2-Gy progeny that was statistically significant when compared with the control group. The results indicate that prezygotic testicular X-ray exposure of paternal animals causes the shift of adenoma-carcinoma sequence towards malignancy in the progeny.

Key words : prezygotic X-ray exposure, susceptibility to cancer, progeny

Introduction

The possibility of germ cell transmission of an increased cancer risk following exposure to ionizing radiation has been reported in a large number of epidemiological and experimental studies.[1-7] However, in most of the previous epidemiological studies only offspring in a certain lifespan have been taken into consideration; namely childhood and adolescence.[8-15] Also, in the experimental studies the interests of the researchers seem to have been in the early life period of offspring, so that the animals were killed at the age of six,[16] six or eight,[17,18] eight or twelve,[3] 13 or 14.519 and 14.56 months, and their results were contradictory.

On the other hand, the possibility of contracting tumour disease increases with age of the individual, not only in humans but also in animals. Furthermore, the offspring will usually be exposed to various environmental carcinogens and/or promoters during their lifetime. An increase of tumour incidence after treatment with exogenous agents in the progeny of parents preconceptually exposed to X-radiation has already been described in laboratory animal models.[16-18] In these investigations, however, only multiplicity of lung tumours was examined. It is generally accepted that, at least in part, different genetic factors play a role with regard to the development of benign or malignant tumours. Therefore, the consequences of germ cell alterations might be revealed in the dignity of tumours induced in offspring.

In the present long-term study the animals were kept for life and the dignity of the lung tumours which occurred was examined to complete the previous investigations on effects of germ cell irradiation to the offspring.

Materials and Methods

The testicles of the 9-week-old male CBA/J mice (Charles River, Sulzfeld, Germany) were X-ray irradiated under ketamine/xylazine narcosis. An X-ray generator for skin irradiation, Philips type RT100 (Philips, Hamburg, Germany) was used, operating at 8 mA and 100 kV with a filter of 1.7 mm of aluminium and 0.2 mm of copper. The total dose of 1 Gy or 2 Gy was administered in two single doses of 0.5 Gy or 1 Gy, respectively, with a 24-hour interval. One week after the X-ray irradiation, the parental males were mated with untreated virgin 12-week-old females of the same strain. The 1-Gy offspring (88 males, 62 females), the 2-Gy offspring (100 males, 93 females) and additional control offspring (83 males, 84 females) were treated once subcutaneously with 0.1 mg/g bw urethane at the age of 6 weeks (Fig. 1). They were housed three per cage in Makrolon Type II cages (350 cm^2) and kept for their entire lifespan under standard laboratory

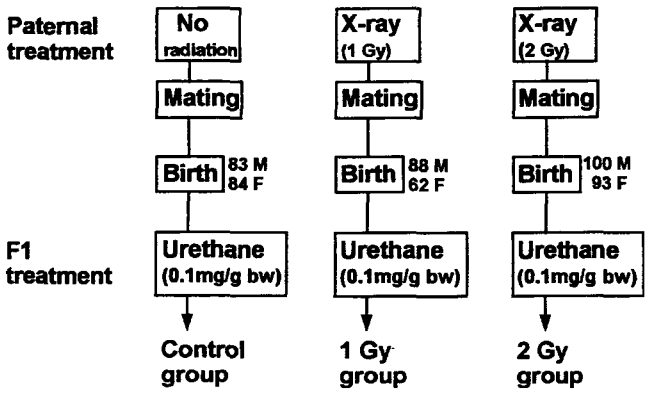

Fig. 1. Experimental design. M=male, F=female.

conditions (room temperature 21 2°C; relative humidity 45-70%; air exchange 15 times/h; 12 h/12 h light/dark cycle). Absorbent softwood (H3/4, Hahn & Co., Kronsburg, Germany) was used as bedding material in the cage. The mice received a pelleted maintenance diet 1324 (Altromin GmbH & Co., Lage, Germany) and tap water *ad libitum*. They were checked daily and weighed weekly; moribund animals were killed with an overdose of CO_2. Uniform methods were used for the autopsy. Lungs were examined by eye and surface tumour nodules counted for each animal. After complete autopsies, all organs were fixed in 10% buffered formalin which was refreshed after 24 hours. Infusion fixation was performed on the lungs. Tissue specimens from each of five lung lobes were embedded in Paraplast PlusTM (Sherwood Medical Co., St. Louis, MO, USA). Three-4 m thick sections were stained with haematoxylin and eosin. All lung sections were carefully examined microscopically. In the histopathological diagnosis, malignant pulmonal tumours were distinguished from the benign according to the common criteria for malignancy, i. e. cellular pleomorphism, enlarged and hyperchromatic nuclei with coarsely clumped chromatin and prominent nucleoli, increased and atypical mitosis, bizarre tumour giant cells, invasion and necrosis. In borderline cases, the adequate publications were referred.[20-24] Tumour diagnoses were documented and evaluated using the Pathology Lexicon Acquisition, Correlation and Evaluation System (PLACES 2000), Version 1 (Apoloco Ltd., Newcastle, England). For the statistics, the two-tailed Fisher's exact test was used.

Results

While the average life expectancy of females varied only slightly between the three groups, the survival rate of the male control offspring was significantly shorter than that of two other male groups (Table 1).

Table 1. Mean survival (weeks)#

Sex	Group		
	Control	1 Gy	2 Gy
Male	82±15	86*±14	87*±14
Female	89±21	86±19	88±19

#Mean S. D.; Student t-test: *$p \leq 0.05$

Table 2. Number of lung tumour-bearing offspring in time windows

		Time Windows (weeks)				
Sex	Group	6-52	53-78	79-104	>104	Total lifespan
Male	Control	1/13 (33%)	12/24 (50%)	26/54 (48%)	2/2 (100%)	41/83 (49%)
	1 Gy	0/3 (0%)	8/20 (40%)	31/60 (52%)	5/5 (100%)	44/88 (50%)
	2 Gy	0/0 (0%)	5/22 (23%)	42/69 (61%)	5/9 (56%)	52/100 (52%)
Female	Control	1/5 (20%)	1/15 (7%)	19/48 (40%)	7/16 (44%)	28/84 (33%)
	1 Gy	1/3 (33%)	1/14 (7%)	17/38 (45%)	4/7 (57%)	23/62 (37%)
	2 Gy	0/3 (0%)	5/26 (19%)	13/43 (30%)	10/21 (48%)	28/93 (30%)

–/–: number of lung tumour-bearing animals/number of dead animals 1 Gy, 2 Gy: paternal treatment

Table 3. Incidence of lung tumours in the progeny

	Male			Female		
Paternal treatment	Control	1 Gy	2Gy	Control	1 Gy	2 Gy
Total animal number	83 (100%)	88 (100%)	100 (100%)	84 (100%)	62 (100%)	93 (100%)
Tumor-bearing animals	41 (49%)	44 (50%)	52 (52%)	28 (33%)	23 (37%)	28 (30%)
amongst: Animal with adenoma only	31 (37%)	27 (31%)	28 (28%)	24 (29%)	17 (27%)	25 (27%)
Animal with carcinoma	10 (12%)	17 (19%)	24 (24%)	4 (5%)	6 (10%)	3 (3%)

The incidence of the lung tumours is presented in Table 2 with time windows of the lifespan showing collectively higher incidence in males than in females. Looking at the relation between tumour occurrence and point of death, a small number of animals actually died within one year after birth (3%), including only three tumour-bearing offspring. The male control animals showed a relatively high incidence of lung tumours in the early lifespan. In contrast to them, the other

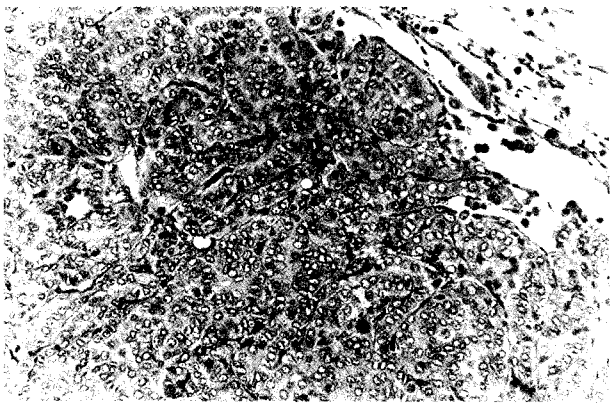

Fig. 2. Well circumscribed adenoma consisting of closely-packed glandular formations with round to oval monomorphic nuclei. H&E, ×25.

five groups demonstrated a tendency rather of primary pulmonary neoplasms occurring in the late period of life.

Table 3 shows the incidences of lung tumours with the determination of their dignity. In the present study, only adenomas and adenocarcinomas were induced in the lungs; other primary lung tumours were not detected.

The lung adenomas (Fig. 2) were round, well circumscribed and up to 3 mm in diameter, showing the slight compression of the surrounding alveolar parenchyma. Not only tubular or solid but also alveolar or papillary growth patterns of tumours were observed. The proliferating epithelial cells included round or oval, relatively monomorphic nuclei with fine chromatin and an abundant eosinophilic cytoplasm. Mitotic figures were mostly rare or absent. Extension of neoplastic tissue into the adjacent bronchioles were occasionally seen. The majority of the adenocarcinomas (Fig. 3) were

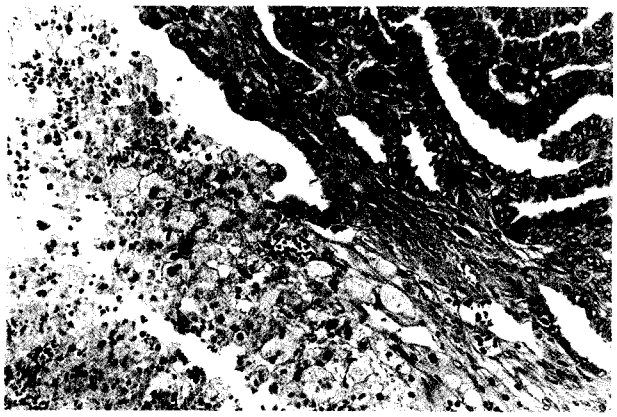

Fig. 3. Papillary area of lung adenocarcinoma with oval to elongated nuclei. Note the necrosis. H&E, ×25.

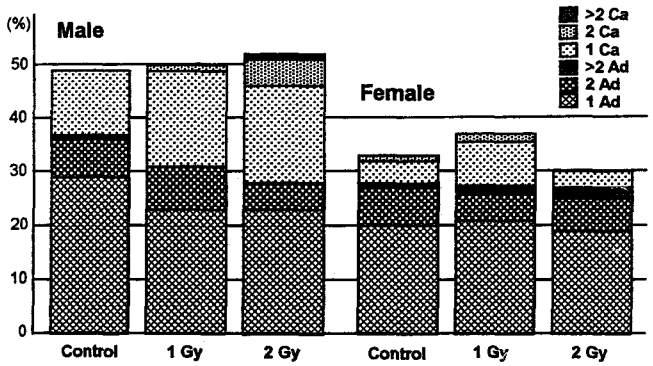

Fig. 4. Tumour incidence and multiplicity in the offspring. Ca=carcinoma, Ad=Adenoma.

irregularly-circumscribed and larger than 3 mm in diameter. The tumours included numerous irregularly-shaped, in some places papillary atypical glandular formations. The glandular spaces were lined by one or two layers of cuboidal to cylindrical epithelial cells which included closely-packed, oval or elongated, moderately pleomorphic nuclei with slightly coarse chromatin and a small amount of cytoplasm. Nucleus/cytoplasm ratio of the atypical epithelial cells was shifted towards the nucleus. Some tumour cells had enlarged nucleoli. Small necrotic areas were occasionally seen within neoplastic nodules.

There were no significant differences between the control, 1-Gy and 2-Gy offspring groups with regard to the tumour incidences in each sex (Table 3).

However, in males the number of carcinoma-bearing offspring increased and the incidence of lung adenomas decreased, depending on the doses of paternal X-ray irradiation. In the 2-Gy group, the increase of the incidence of lung carcinomas was tightly below statistical significance ($p=0.055$) compared with the male control group. Fig. 4 elucidates these findings graphically. In addition, Fig. 4 shows that in the male 1-Gy offspring group a few animals had two adenocarcinomas of the lung, and in the 2-Gy group the number of the offspring with double carcinomas increased and some animals also developed multiple lung carcinomas, although in the male conrol offspring there were animals with multiple adenomas but not multiple carcinomas.

Hereby, the incidence of multiple lung carcinomas in the 2-Gy group was statistically significant ($p=0.033$).

In females, similar clear-cut dose-dependent findings were not recognized. However, in the 1-Gy offspring group the incidence of adenocarcinomas indicated a small increase (Table 3).

Discussion

The average survival rates of our animals were within the limits of variability published in the literature, between 75 and 107 weeks.[25] The low dose of urethane given to each offspring seems to have had no life-shortening effect. The cause of the short survival rate in the control males could be spontaneous tumour occurrences in the relatively early life period of this group; among them carcinomas (data not shown). Otherwise, our results conform with the general acceptance that the majority of individuals develop tumours in their late lifespan. The reasons might be the long latency period of tumour disease and accumulation of neoplasm-related genetic alterations.[26,27] Accordingly, keeping animals for their entire lifespan is necessary to obtain consolidated results in tumorigenesis studies.

Our results indicate that the prezygotic X-ray exposure of male parental animals caused the shift of adenoma-carcinoma sequence towards malignancy in the male progeny. The question is, what kinds of gene alterations were induced in paternal germ cells by the X-ray exposure and transmitted to the offspring. Up to now it has been unclear which gene or genes are responsible for the progression from adenoma to adenocarcinoma in the lungs of mice.

The following possibilities are conceivable.

1. Through the effects of the X-ray exposure on the genes related to regulation of carcinogen metabolism (cytochrome P450 genes and glutathone-S-transferase genes), the offspring became good metabolisers. 2. Being caused by X-ray effects on the genes related to defective processes of DNA repair (mismatch repair genes), urethane-induced miscoding DNA adducts could not be repaired correctly. 1. and 2. resulted in increased deployment of the carcinogenic effect of urethane. 3. The testicular X-ray exposure inactivated suppressor genes, for example p53, and this genetic information was transmitted to the offspring, because in the human colon carcinoma, the inactivation of the p53 suppressor gene is necessary for the progression from adenoma to intramucosal carcinoma.[28] According to Festing and his co-worker,[29] at least four genes are associated with susceptibility to urethane-induced pulmonary adenomas in mice. So, it is possible that 4. multiple gene alterations induced by paternal X-ray exposure also played a role in the progression from adenoma to carcinoma in the progeny.

Chen and his co-worker[30] showed in their publication that the K-ras gene contains regulatory elements associated with mouse lung tumour susceptibility. In humans, the point mutation of the K-ras oncogene is an important gene alteration particularly with regard to the progression to lung adenocarcinoma.[31] However, in our investigation, the K-ras oncogene does not seem to have been altered by X-ray since, in the treated groups, the incidences of the lung tumours did not increase significantly, and the genetic analysis of the tumours from the present study showed that the K-ras mutations play a role in the early steps of mouse lung tumorigenesis but not in the progression from adenoma to carcinoma.[32] Furthermore, the investigations of Kawano et al.[33,34] using B6C3F1 and A/J mice resulted in the same conclusions as those of Cazorla and her co-worker.

It is known that germline mutations at hypervariable mice minisatellite loci can be induced by ionizing radiation.[35-37] However, it is not clear whether these mutations at minisatellite loci are the cause of the phenomenon of our study.

In addition, two similar investigations, using 363 and 324 offspring, with different mating timepoints were carried out; i.e. 3 and 9 weeks after paternal testicular irradiation, respectively. Other experimental conditions were exactly the same as the one-week study described. These two additional studies did not show any significant differences between treated and control offspring with regard to the occurrence of lung carcinomas. This observation underlines the sensitivity of the spermatozoa to radiation and is in accordance with Nomura's works,[17,38] which showed a higher sensitivity of post-meiotic germ cells (spermatozoa and spermatids) than of pre-meiotic spermatogonia.

From another point of view it should also be considered that the germline alterations were completely repaired before the mating 3 or 9 weeks after testicular irradiation.

In conclusion, prezygotic testicular X-ray exposure of paternal animals causes the shift of adenoma-carcinoma sequence towards malignancy in the progeny. Further suitable investigations should be carried out to clarify what kinds of vertical transmitted germline alterations are responsible for this phenomenon. The spermatozoa stage seems to be the most sensitive to irradiation compared with the other spermatogenic stages.

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