

Case Report

## Two Cases of Metastatic Parathyroid Carcinoma in Male C3H Mice Following Irradiation

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**Abstract:** White nodules were observed in the thyroid in two male C3H mice (at 99 and 122 weeks of age) exposed to fast neutrons at the age of 8 weeks. Histopathologically, in both cases, tumors were developed in the region corresponding to the parathyroid gland, and the tumor cells were arranged in a solid sheet or nest-like structures. Necrosis, cell debris and/or hemorrhage were sometimes seen in the center of the tumor structures. Tumor cells were small and uniform with scanty cytoplasm, cell margins were indistinct, and basally located tumor cells were aligned along the vascular stroma. Mitotic figures were frequently observed. Metastasis to the renal cortex was observed in both cases. These cases were diagnosed as parathyroid carcinoma. A parathyroid tumor is an extremely rare endocrine tumor in mice, regardless of whether the tumor is spontaneous or experimentally induced. These cases may have been induced by neutron-exposure; however, how radiation induces parathyroid carcinoma in mice is not clear. (DOI: 10.1293/tox.2013-0018; J Toxicol Pathol 2013; 26: 413–417)

**Key words:** parathyroid carcinoma, C3H, mouse, neutron, metastasis

It is known that parathyroid tumors in humans, dogs and cats cause hyperparathyroidism and that there are endocrine tumors that occur secondary to hyperparathyroidism. On the other hand, naturally occurring parathyroid tumors are rare in mice, and there have been few reports of parathyroid tumors induced by radiation or chemicals in mice. In this report, we describe two cases of parathyroid carcinoma observed in C3H/HeNrs mice exposed to fast neutrons.

The two cases were found in a radiation carcinogenesis study with the aim of radiation cancer risk analysis of fast neutrons. The animals used were bred at our institute. They were housed in an animal room controlled at temperature of  $23 \pm 1^\circ\text{C}$  and a relative humidity of  $55 \pm 5\%$  with a 12 hour light-dark cycle, were fed standard commercial laboratory diet (MB-5, Funabashi Farm Co., Tokyo, Japan), and were given chlorinated water *ad libitum*. A total of 2660 mice were assigned to 13 groups: a non-irradiated control group containing 57 mice; 6 fast neutron-irradiated groups (0.05, 0.1, 0.2, 0.5, 1 and 2 Gy) containing 246, 260, 211, 158, 157 and 156 mice, respectively; and 6 gamma ray-irradiated groups (0.2, 0.5, 1, 2, 3 and 4 Gy) containing 265, 262, 211, 162, 157 and 158 mice, respectively. All the animal experiments were carried out with permission and under regula-

tion of the Institutional Committee for Animal Safety and Welfare of the National Institute of Radiological Sciences. Neutrons were generated from the NIRS cyclotron using the deuterium-Beryllium reaction. The estimated forward neutron spectra established a peak energy of 10 MeV. Dosimetry was conducted using an ion chamber filled with tissue equivalent gas, and the dose of neutrons in this study was expressed as *kerma* (Gy). The contamination of gamma-rays was estimated to be about 5% of the neutron dose. Exposure to Cs-137 gamma rays was conducted with Gammacell (Nordion Inc., Ottawa, ON, Canada). The mean dose rate was 0.15 Gy/min for neutrons and 0.65 Gy/min for gamma-rays, respectively. After the irradiation, the animals were observed throughout their lives. One mouse was skinny and anemic and became moribund; it was sacrificed 637 days after irradiation of 1 Gy of fast neutrons (Case 1). The other mouse had tachypnea and became moribund. It was sacrificed 798 days after irradiation of 0.1 Gy of fast neutrons (Case 2). Macroscopically, Case 1 had a white nodule in the left thyroid, three white nodules in the lungs, two white nodules in the liver, two white nodules in the kidneys and eight white nodules in the spleen. In addition, Case 1 had dark red nodules in the livers, and small white nodules in the adrenal glands. Case 2 had a white nodule in the right thyroid, dark red nodules in the liver and a small white nodule in an adrenal gland.

All the tissues routinely collected were weighed, fixed with 10% neutral-buffered formalin and subjected to histopathological examination. Each paraffin-embedded section was stained with hematoxylin-eosin (HE). Immunohisto-

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chemistry was performed as follows. The primary antibodies used were monoclonal anti-PCNA antibody (Clone PC10, 1:10, DAKO Tokyo, Japan), polyclonal anti-PTH antibody (pre-diluted, Lab Vision, Fremont, CA, USA), polyclonal anti-p27 antibody (pre-diluted, GeneTex, Irvine, CA, USA) and monoclonal anti-Cyclin D1 antibody (Clone DCS-6, pre-diluted, Progen Biotechnik GmbH, Heidelberg, Germany). Deparaffinized sections were incubated with the primary antibodies at 4°C overnight, and this was followed by peroxidase-labeled secondary antibody reactions at room temperature for 30 min using Histofine Simple Stain MAX-PO (MULTI) (Nichirei, Tokyo, Japan). Finally, the positive reaction was visualized with 0.02% 3,3'-diaminobenzidine (DAB) and 0.02% hydrogen peroxide in a Tris-HCl buffer, and the sections were counterstained with hematoxylin.

Histopathologically, in both cases, tumors were developed in the region corresponding to the parathyroid gland, and the tumor cells were arranged in a solid sheet or nest-like structures. Necrosis, cell debris and/or hemorrhage were sometimes seen in the center of the tumor structures (Fig. 1). Tumor cells were small and uniform with scanty cytoplasm, cell margins were indistinct, basally-located tumor cells were aligned along the vascular stroma, and mitotic figures were frequently observed (Fig. 2). Metastasis to the renal cortex, liver, spleen, lungs, endocardium, bone marrow and stroma surrounding accessory reproductive glands was observed in Case 1, and metastasis to the renal cortex was observed in Case 2 (Figs. 3 and 4). Immunohistochemistry showed that in both cases, the tumors had numerous PCNA positive cells (Fig. 5a) and were PTH negative (Fig. 6a), while normal parathyroid epithelial cells were PCNA negative (Fig. 5b) and PTH-positive (Fig. 6b). The tumor cells were p27 positive (Fig. 7) and Cyclin D1 negative (Fig. 8).

In addition, atrophy of seminiferous tubules and subcapsular cell hyperplasia in adrenal glands were observed in Case 1. Thyroid adenoma, hepatocellular adenoma, Harderian gland adenoma and subcapsular cell hyperplasia in adrenal glands were seen in Case 2. No abnormal changes were seen in the digestive tract in both cases. In this radiation carcinogenesis study, no proliferative lesions were seen in mice other than these two animals.

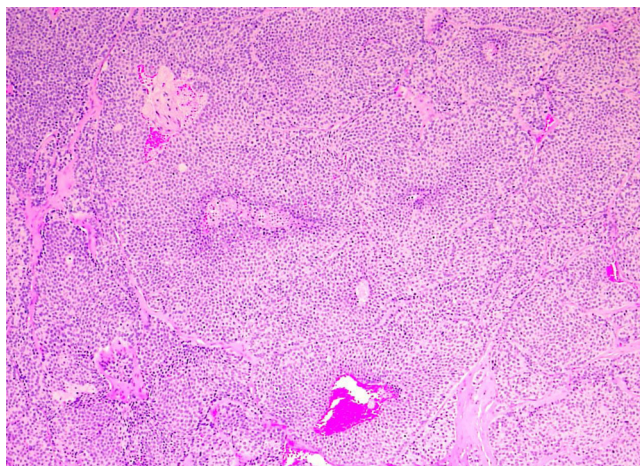
Based on the above-mentioned findings, the white nodules in the thyroid in these cases were diagnosed as parathyroid carcinoma. Considering the characteristics of parathyroid carcinoma in mice based on the two cases in the present study, metastasis to the kidneys may be common, and hematogenous systemic metastasis can also occur. In humans, it is known that parathyroid carcinoma shows gross infiltration into adjacent thyroid, nerve, muscle or esophagus tissue or cervical node metastases<sup>1</sup>. Metastases occur via both lymphatic and hematogenous routes, and human parathyroid carcinoma showed metastases to cervical nodes (30%), the lung (40%) and the liver (10%), with lower incidences of metastases to the bone, pleura, pericardium and pancreas<sup>1</sup>. On the other hand, we could not find any literatures reporting metastasis of parathyroid carcinoma in mice

and rats. Regarding the pathogenesis of the present cases, it is unlikely that renal or nutritional secondary hyperparathyroidism caused the tumors, because there were no histological alterations in the glomeruli and tubulointerstitium of the kidney or in the gastrointestinal tracts in these cases and because the contralateral parathyroid gland, which was tumor free, was normal in these cases.

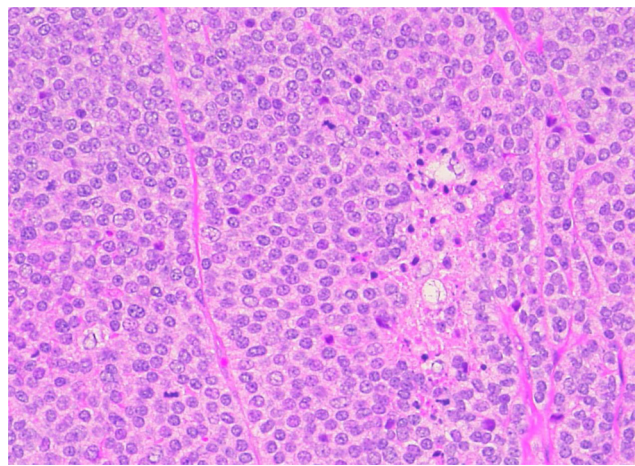
The present cases were considered to be extremely rare in male mice. In the previous reports on parathyroid tumors in mice, there were only 8 cases including two cases of adenoma, one in a 24-month-old female C3H/HeB mouse and one in a 24-month-old female C3H mouse<sup>2</sup>, two cases of adenoma in female CDI mice, one at 111 weeks of age and one at 103 weeks of age<sup>3</sup>, and three cases of adenoma and a case of carcinoma in female B6C3F1 mice in NTP carcinogenicity studies<sup>4</sup>.

On the other hand, it is reported that parathyroid adenoma was induced in eleven out of forty-five female rats by local irradiation with a single parathyroid dose of about 6.5 Gy<sup>5</sup> and that the occurrence of parathyroid tumors increased in the patients who received head and neck radiation therapy<sup>6,7</sup>. The fast neutrons used for irradiation in the present study are thought to have larger biological effects than gamma-rays or X-rays. In addition, whereas parathyroid tumors of rodents have been reported in females so far, it is interesting that parathyroid carcinoma was seen in males.

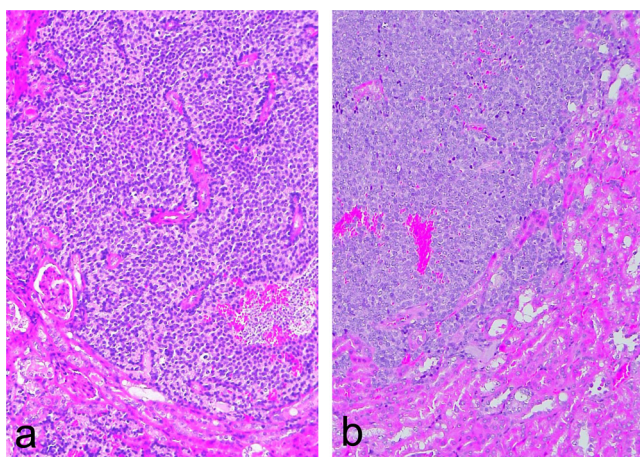
It is reported that alterations of an oncogene, cyclin D1, and a tumor suppressor gene, MEN 1, were observed in parathyroid adenoma in humans<sup>8</sup>. Overexpression of cyclin D1 has been found in parathyroid carcinomas, adenomas and hyperplasias in humans<sup>9</sup>, and parathyroid hormone gene regulatory region-cyclin D1 (PTH-cyclin D1) mice were found to develop abnormal parathyroid cell proliferation with clinical symptoms of hyperparathyroidism<sup>10</sup>. These results indicate that cyclin D1 has an important role in parathyroid tumorigenesis. In the present cases, overexpression of cyclin D1 was not detected by immunohistochemical analysis. Although PCNA-positive tumor cells and mitosis were frequently seen in the present cases, absence of cyclin D1-immunoreactivity in the tumor cells might indicate that there might be some abnormalities in cyclin D1 expression. Together with the results of PTH immunostaining, this may indicate that the present cases may not involve the same alterations in cyclin D1 and PTH, which are well documented in human parathyroid tumors, in their pathogenesis. In addition, loss of p27-immunoreactivity was reported in human parathyroid carcinoma cases<sup>11</sup>, and it is reported that mice lacking both alleles of p27<sup>Kip1</sup> developed parathyroid adenoma or hyperplasia<sup>12</sup>. Since p27 is a cyclin-dependent kinase inhibitor that regulates the cell cycle and blocks cell proliferation<sup>11</sup>, low levels of the p27 protein might correlate with the malignancy of many tumors. However, the present cases showed positive p27-immunoreactivity in the tumor cells. Though we cannot surmise the meaning of this result, abnormal p27 protein expression may possibly be related to that. In any case, the discrepancies in the immunoreactivities of Cyclin D1 and p27 between human parathyroid car-



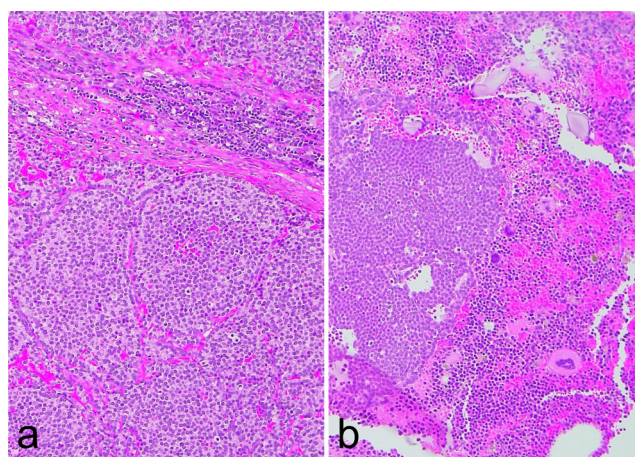
**Fig. 1.** The parathyroid showed a solid sheet or nest-like structure, and necrosis in the center of the nest-like tumor structures was also seen (Case 1, original magnification,  $\times 10$ ).



**Fig. 2.** Tumor cells were small and uniform with scanty cytoplasm, cell margins were indistinct, and basally located tumor cells were aligned along the vascular stroma, and mitotic figures were frequently observed (Case 2, original magnification,  $\times 40$ ).



**Fig. 3.** Metastases into the renal cortex. Infiltration and proliferation of tumor cells were observed in the renal tubular interstitium and around the glomerulus. a: Necrosis was found in Case 1 (original magnification,  $\times 20$ ). b: A solid growth pattern and numerous mitotic figures were seen in Case 2 (original magnification,  $\times 20$ ).



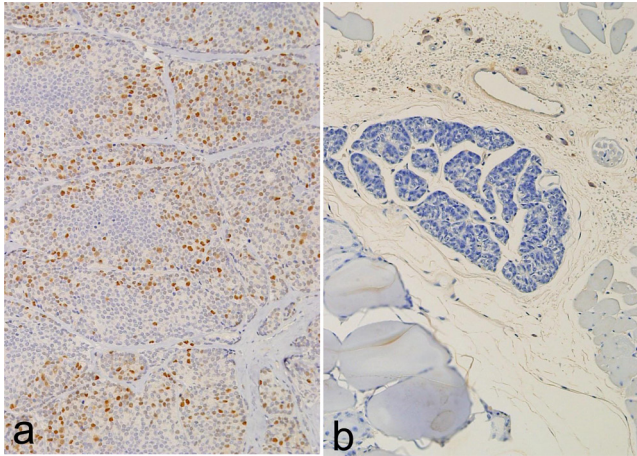
**Fig. 4.** a: Spleen from Case 1. The spleen was mostly replaced by tumor cells. (original magnification,  $\times 20$ ). b: Bone marrow from Case 1. Nodular proliferation of metastatic tumor cells was observed (original magnification,  $\times 20$ ).

cinomas and the present mouse cases are unknown at present. Examinations of the alterations in oncogenes and tumor suppressive genes may provide useful information concerning the mechanism of murine parathyroid carcinoma.

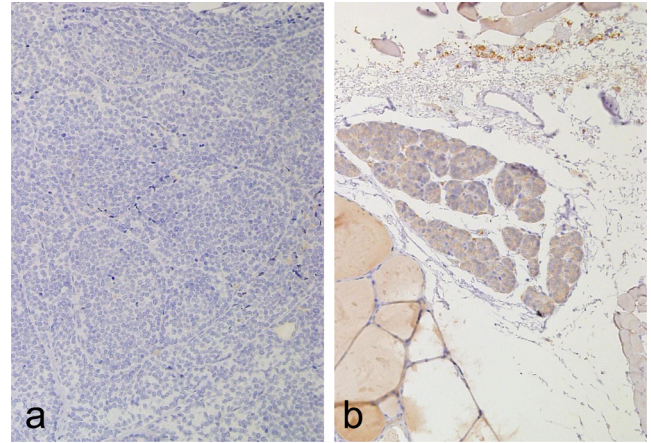
Since parathyroid tumors are rare and clinical symptoms of hyperparathyroidism are not well understood in mice, it is unclear whether the murine parathyroid carcinoma is hormonally functional or not. In humans, patients with parathyroid carcinoma typically have severe primary hyperparathyroidism with marked hypercalcemia and elevated parathyroid hormone levels<sup>1</sup>, and human parathyroid adenomas and carcinomas are positive for PTH immunos-

taining<sup>13</sup>. In our search of the literature, we could not find any reports that described results concerning PTH immunoreactivities in parathyroid tumors in mice and rats. On the other hand, it was assumed that two mouse parathyroid adenomas reported by Lewis and Cherry<sup>3</sup> and two rat parathyroid adenomas reported by Snell<sup>14</sup> were non-functional tumors<sup>3,14</sup>, since neither rat had any pathologic changes in the kidneys or bone indicative of deranged calcium metabolism. The present cases showed negative PTH immunoreactivity and no osteoporosis lesions, so it is thought that these were cases of nonfunctional parathyroid cancer.

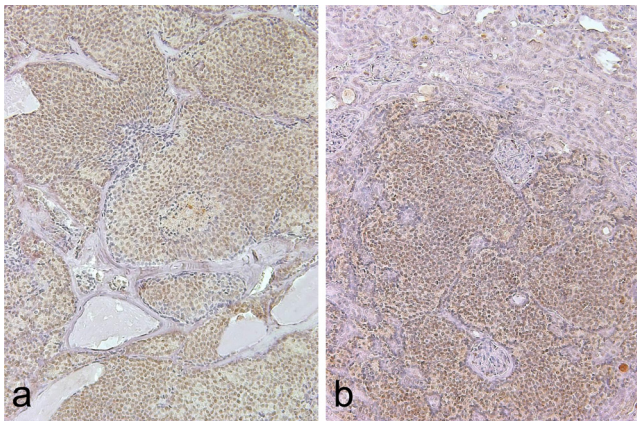
In conclusion, two cases of parathyroid carcinoma were found in male C3H mice irradiated with fast neutrons. The tumors were characterized by a solid sheet or nest-like



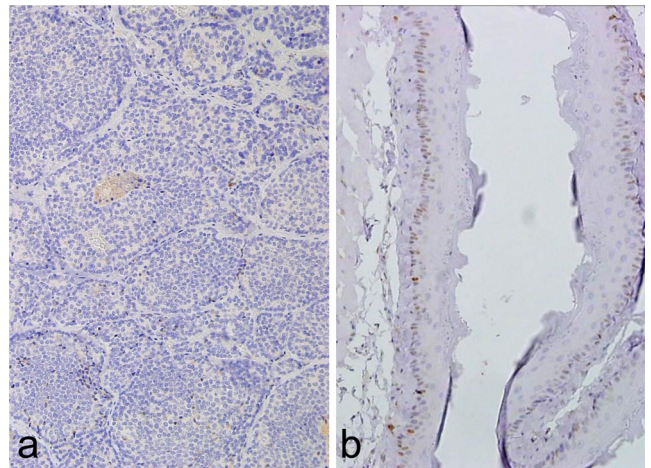
**Fig. 5.** PCNA immunostaining. a: Many PCNA-positive tumor cells were seen in Case 1. Tumor cells in the center of the nodule were usually negative for PCNA-staining (original magnification,  $\times 20$ ). b: No PCNA-positive cells were seen on the opposite side of the parathyroid gland (normal parathyroid gland) of Case 2 (original magnification,  $\times 20$ ).



**Fig. 6.** PTH immunostaining. a: Weak PTH-positive cells were seen in the normal parathyroid gland of Case 2 (original magnification,  $\times 20$ ). b: PTH-negative staining was observed in the neoplastic parathyroid gland of Case 2 (original magnification,  $\times 20$ ).



**Fig. 7.** p27 immunostaining. a: p27-positive staining was observed in the primary tumor (Case 1, original magnification,  $\times 20$ ). b: The tumor cells of a metastatic focus in the kidney were also p27positive (Case 1, original magnification,  $\times 20$ ).



**Fig. 8.** Cyclin D1 immunostaining. a: Cyclin D1-negative staining was observed in the tumor (Case 1, original magnification,  $\times 20$ ). b: Cyclin D1-positive cells were seen in the basal layer of the esophagus (Case 2, original magnification,  $\times 20$ ).

growth pattern sometimes accompanied by necrosis or hemorrhage in the center of the tumor structure; the basally-located tumor cells were aligned along the vascular stroma, and metastasis to the kidney was commonly seen. Although the tumor had a high proliferative capability, Cyclin D1, which was reported to be upregulated in human parathyroid carcinoma, was negative. Hyperparathyroidism might not be involved in the pathogenesis of these cases. The present cases might have been induced by irradiation with fast neutrons, but the details of the pathogenesis are still obscure. Parathyroid carcinoma in mice is a rare tumor; so it is necessary to accumulate further cases to clarify the natures of these tumors.

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