Case Report

A case report of a metastatic yolk sac carcinoma in the pulmonary artery of a young female Sprague-Dawley rat

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Abstract: Yolk sac carcinoma is an extremely rare tumor in rats and is usually found in the genital system of aged animals. We encountered a yolk sac carcinoma in the pulmonary artery of an 18-week-old female Sprague-Dawley rat. In a repeated dosing toxicity study (once weekly for 4 weeks, intraperitoneal), this rat was unexpectedly found dead on the 55th day after the final administration of the test article. At necropsy, grayish white nodules were found on the lung surface. Histopathologically, tumor emboli were observed in the trunk and branch of the pulmonary artery. Tumor cells with slightly basophilic vacuolated cytoplasm and large vesicular nuclei formed nests or clusters and were embedded in a homogenous eosinophilic and periodic acid-Schiff reaction positive matrix. The tumor cells and matrix were immunoreactive for laminin. The embolic tumor resembled yolk sac carcinoma showing a parietal pattern in rodents. Although the primary site was unknown, the tumor was considered to be a metastatic yolk sac carcinoma. (DOI: 10.1293/tox.2016-0025; J Toxicol Pathol 2016; 29: 269–273)

Key words: yolk sac carcinoma, pulmonary artery, lung, rat, young, female

Yolk sac carcinoma is a very rare malignant tumor in rodents which is considered to be a variant of germ cell tumors and microscopically resembles a yolk sac, an extraembryonic membrane. The incidence of yolk sac carcinoma in rats is extremely low, with only 1 case occurring in more than 40,000 female F344 rats used in previous carcinogenicity studies[1]. There have been some other reports of yolk sac carcinomas that developed in the ovary or uterus in aged female rats[2–4]. Nakazawa and colleagues reported an instance of testicular yolk sac carcinoma in an aged Sprague-Dawley (SD) rat[5]. Yolk sac carcinoma can also be experimentally induced in rats by surgical procedures, such as fetectomy with or without additional injection of mouse sarcoma virus[6, 7], puncture of the pregnant uterus wall in mid gestation[8], and implantation of an embryo under the kidney capsule of a syngeneic rat[9]. As described above, yolk sac carcinomas in rats are generally encountered in older animals and observed in genital systems. Here, we report a case of metastatic yolk sac carcinoma in the pulmonary artery of a young female rat.

The female SD rat was obtained from Charles River Laboratories Japan, Inc. (Kanagawa, Japan) at 5 weeks of age. The rat was housed alone in a stainless-steel cage in an air conditioned animal room controlled at a temperature of 24 ± 3°C with a humidity of 50 ± 20% and a 12 hour light/dark cycle, was fed a solid diet (CRF-1; Oriental Yeast Co., Ltd.), and was provided with drinking water *ad libitum*. The toxicity study was approved by the Institutional Animal Care and Use Committee and was performed in accordance with the animal welfare bylaws of Drug Safety Research Laboratories, Shin Nippon Biomedical Laboratories, Ltd. After a 2-week acclimation period, the rat was allocated to the high dose group in the study, and the test material (a biodegradative material) was intraperitoneally administered via a paramedian incision of 1 to 2 cm once a week for 4 weeks under anesthesia with intramuscular injection (0.2 mL/kg, each) of medetomidine hydrochloride and ketamine hydrochloride.

On the 55th day of the recovery period after the final administration of the test material (at 18 weeks of age), the rat, which had not exhibited any abnormalities in its general condition, body weight, or food consumption during the study, was unexpectedly found dead. At necropsy, small grayish white nodules were observed on the surface of the lung (Fig. 1). The heart weight was approximately twice than the other rats in the study, which were sacrificed at the end of recovery period (at 23 weeks of age). At necropsy, the right ventricular wall exhibited hypertrophy. Some of the remaining test material was observed in the abdominal
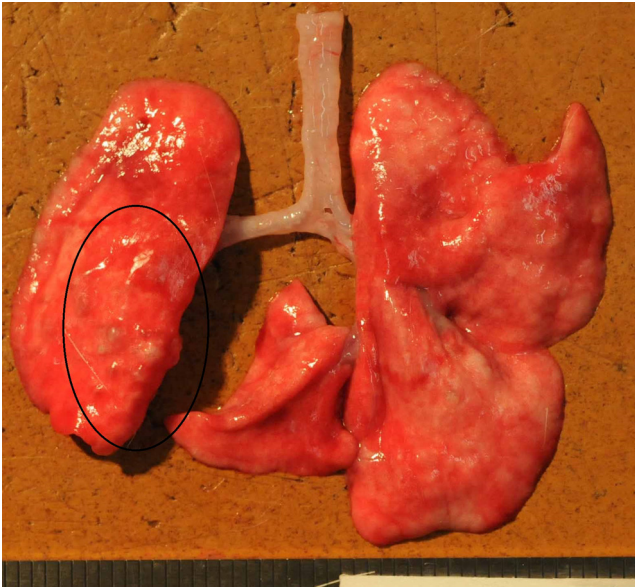
Fig. 1. Gross appearance of the lung. Some grayish white nodules (enclosed by a circle) were observed on the surface of the left lobe.

cavity at necropsy, however, this finding was normally observed following the intraperitoneal administration of this material.

The principal organs and tissues from the whole body, including the lung and heart with the pulmonary artery, were fixed in 10% phosphate-buffered formalin and embedded in paraffin. Paraffin sections were stained with hematoxylin and eosin. Sections from the lung and heart were processed with periodic acid-Schiff (PAS) reaction and phosphotungstic acid-hematoxylin (PTAH) stain. Immunohistochemical staining was performed in the sections from the lung and heart with anti-human cytokeratin mouse monoclonal antibody (AE1/AE3, ×1, Dako North America, Inc., Carpinteria, CA, USA), anti-laminin rabbit polyclonal antibody (×5,000, Dako Cytomation, Inc. Denmark), and alpha-fetoprotein (AFP) antibody (C-19, ×100, Santa Cruz Biotechnology, Inc., Dallas, TX, USA) and the sections were subjected to a subsequent chromogenic reaction with the streptavidin-biotin complex method.

In the histopathological examination of the lung, multiple intravascular emboli were observed in the branches of the pulmonary artery. Emboli were present in the left and right (posterior) lobes of the lung. Large and small emboli appeared to be occlusive, with close approximation or adherence to the vascular endothelium, and were considered to be the cause of death via obstruction of blood flow (Fig. 2A). A similar embolus was also seen in the trunk of the pulmonary artery (Fig. 2B). In the heart, fibrosis and mononuclear cell infiltration were observed in the right ventricular wall and interventricular septum, respectively. In the liver, congestion was observed. Together with the heart weight increase and right ventricular hypertrophy, these histopathological findings in the heart and liver suggested circulatory

disturbance.

The intravascular emboli were mainly characterized by the presence of small nests or clusters of undifferentiated cells embedded in a prominent homogenous eosinophilic matrix (Fig. 2C, 2D). In a few areas, it appeared as if similar cellular foci were growing within the lung parenchyma, which is indicative of invasion (Fig. 2E). The appearances of multiple emboli in the trunk and branches of the pulmonary artery and the evidence of invasion were indicative of metastasis of a neoplastic tumor.

The predominant cell type within the tumor emboli appeared as irregularly shaped slightly basophilic cells with vacuolated cytoplasm and large vesicular nuclei containing one or two nucleoli. Tumor cells within the nests or clusters appeared closely arranged and attached to each other. Infrequent hyaline droplets were present in tumor cells, suggesting that the eosinophilic matrix around the cell clusters was produced by the tumor cells (Fig. 2D). Individual cell necrosis of tumor cells was present throughout the neoplastic process. Mitoses were evident but few (Fig. 2E).

From the appearance of the H&E sections as described above, we suspected yolk sac carcinoma and conducted a PAS reaction and immunohistochemistry to cytokeratin, laminin, and AFP. The characteristic eosinophilic matrix was intensively positive for the PAS reaction and laminin (Fig. 3A, 3B). The cytoplasm of tumor cells was intensely positive for laminin and weakly and partially positive for AFP and cytokeratin (Fig. 3B, 3C, 3D), respectively. All of the morphological, histochemical, and immunohistochemical findings were highly comparable to the previously reported cases of yolk sac carcinoma in rodents[2, 4, 5, 10]. PTAH staining was performed to differentiate the possibility of thrombi and produced a negative result.

In the necropsy and histopathological examination, no other neoplastic lesions were identified in any other organ, including female reproductive organs, despite additional sectioning of the ovaries and uterus after the regular histopathological examination. Therefore, the organ in which the tumor originated was unknown.

Histologically, yolk sac carcinoma in rodents is described as having two components that resemble a parietal yolk sac and visceral yolk sac. The parietal component is characterized by a cluster of polygonal or cuboidal tumor cells with a surrounding PAS-positive eosinophilic matrix. The visceral component consists of cylindriform tumor cells arranged in a papillary pattern without an eosinophilic matrix, and sometimes a thin basement membrane between the endoderm and the mesenchyme can be observed[2]. In the present case, a visceral pattern was not obvious, and the majority of the components showed a parietal pattern; however, the results of PAS reaction and immunohistochemistry for laminin strongly supported the diagnosis of this tumor as a yolk sac carcinoma. Cytokeratin has also been reported to be positive in rat yolk sac carcinoma cells[4]. Increased levels of AFP were detected in the sera of rats or humans bearing yolk sac carcinomas. Using immunohistochemistry, it was found that AFP was secreted by the yolk sac carcinoma cells
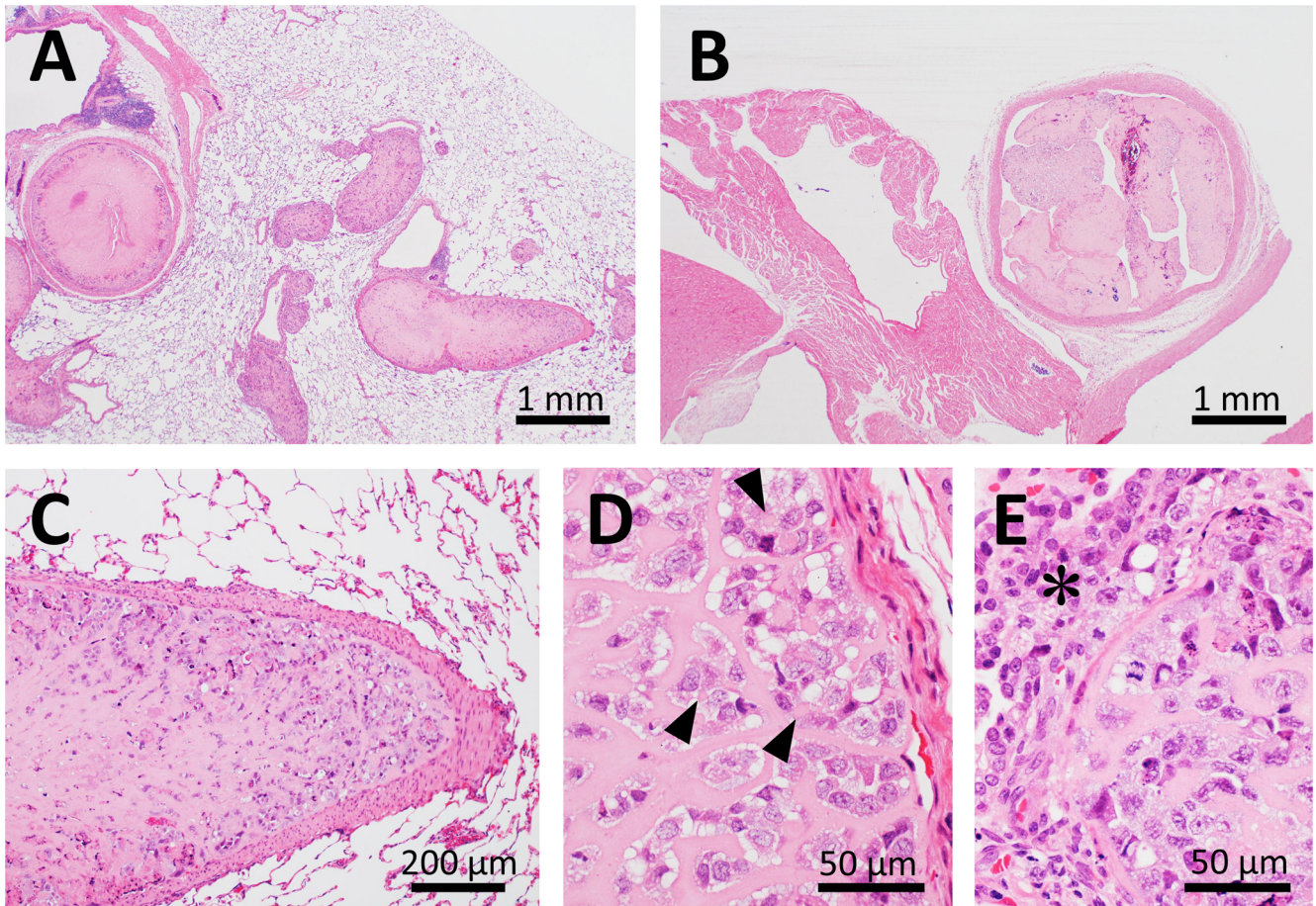
Fig. 2. Photomicrographs of the emboli. Hematoxylin and eosin stain. A) Multiple emboli were observed in the branches of the pulmonary artery. B) An embolus was also observed in the origin of the pulmonary artery. C) Nests or clusters of the neoplastic cells were surrounded by an eosinophilic matrix. D) The neoplastic cells exhibited basophilic vacuolar cytoplasm with large vesicular nuclei. Cytoplasmic hyaline droplets were evident (arrow heads). E) Necrosis and mitosis were present in the neoplastic cells. The presence of tumor cells in the extravascular space indicated invasion (asterisk).

displaying the visceral type[10, 11]. Thus it was reasonable that AFP was not intensely observed in the present case, which exhibited a parietal pattern.

This case is interesting and unique because of the animal's younger age and the lack of gross or microscopic evidence of a primary tumor. Pirak and colleagues[12] reported that choriocarcinoma, another germ cell tumor variant, developed in the cervical lymph nodes and lung (pleural surface) of a 15-week-old SD rat; however, its pathogenesis was considered to be different from our case, in which tumor cells were only observed in the pulmonary artery. Although it has not been published in the English literature, Kajikawa and colleagues[13] reported at the annual meeting of the Japanese Society of Toxicologic Pathology in 2006 that metastatic yolk sac carcinoma was found in the right ventricle and lung in a 10-week-old female SD rat. Kajikawa's case is similar to the present case in two aspects, namely, the presence of tumors in the right ventricle and/or pulmonary artery and the undetected origin of the tumors. Given the intravascular growth pattern of this tumor, it should be considered that a

primary tumor in the gonads or an extragonadal region become necrotic and resorbed following metastasis.

In contrast to rodents, there are some reports indicating that yolk sac carcinoma occurred in the lung in humans with no evidence of a gonadal lesion[14–18]. The origin of pulmonary germ cell tumors in humans is not well known, but it is possible that primordial germ cells remain in the region of the mediastinum or lungs at the time of migration along the gonadal ridge during embryogenesis and become the tumor "seed", eventually developing extragonadal germ cell tumor[17–19]. The origin of the pulmonary germ cell tumors in these human cases is more similar to that in Pirak's report[12] than that in the present case. When a germ cell tumor occurs in the rat lung, we have to consider the following two possibilities for its origin: metastasis from the gonadal or extragonadal primary tumor and a locally occurred tumor which was developed from a migrated primordial germ cell.

It was surprising that the animal did not show abnormal clinical signs or a decrease in body weight prior to death, despite a number of emboli in the pulmonary artery. It was
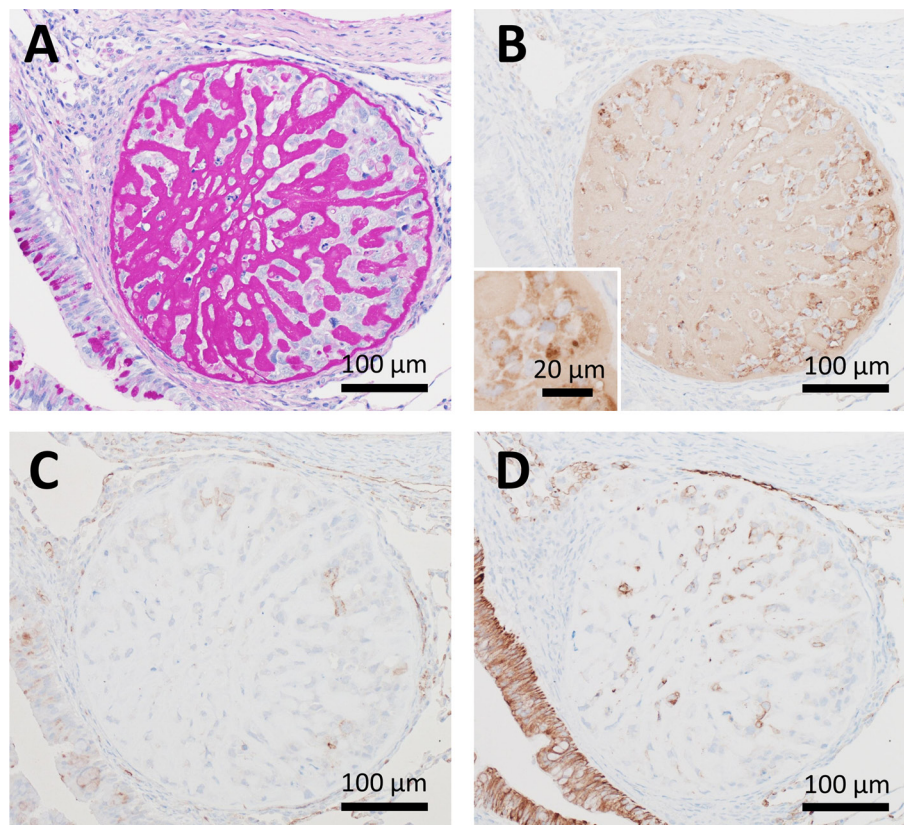
Fig. 3. Photomicrographs of PAS reaction and immunohistochemistry. A) The extracellular matrix showed a strongly positive PAS reaction. Some PAS-positive droplets were evident in the cytoplasm of the tumor cells. B) The extracellular matrix and tumor cells (see insert) were positive for laminin. C and D) A few tumor cells were slightly positive for alpha fetoprotein (C) and cytokeratin (D).

possible that a large embolus developed in the right ventricle and dropped into the trunk of the pulmonary artery, delivering the fatal blow.

The present case was considered to be a spontaneous tumor for the following reasons: there were no other reports of chemically induced yolk sac carcinoma, there was only one case in the study, and it was too early after administration of the test material, which has no genotoxic potential, for it to be the cause of induction and development of the tumor.

In conclusion, this was a very rare case of yolk sac carcinoma that developed in and embolized the pulmonary artery of 18-week-old female SD rat. The tumor cells showed characteristics of a parietal pattern of yolk sac carcinoma, such as cluster formation with a surrounding PAS- and laminin-positive, eosinophilic matrix. To the best of our knowledge, this is the first report of a metastatic pulmonary yolk sac carcinoma in the pulmonary artery without a clear primary site in a rat in the published English literature.

Disclosure of Potential Conflicts of Interest: The authors declare that they have no conflicts of interest with respect to the research, authorship, and/or publication of this article.

References

1. Alison RH, and Morgan KT. Ovarian neoplasms in F344 rats and B6C3F1 mice. Environ Health Perspect. **73**: 91–106. 1987. [Medline] [CrossRef]
2. Sobis H. Yolk sac carcinoma, rat. In: Monographs on the Pathology of Laboratory Animals – Genital System. TC Jones, U Mohr, and RD Hunt (eds). Springer-Verlag, Berlin. 127–133. 1987.
3. Cooper TK, Dumpala PR, and Whitcomb TL. Diagnostic exercise: ascites, abdominal masses, and diffuse peritoneal nodules in a rat. Vet Pathol. **51**: 659–662. 2014. [Medline] [CrossRef]
4. Sakamoto A, Yamaguchi Y, Yamakawa S, Nagatani M, and Tamura K. Highly metastatic ovarian yolk sac carcinoma in a rat. J Toxicol Pathol. **24**: 81–85. 2011. [Medline] [CrossRef]
5. Nakazawa M, Tawaratani T, Uchimoto H, Kawaminami A, Ueda M, Ueda A, Iwakura K, Sumi N, and Kura K. Testicular yolk sac carcinoma in an aged Sprague-Dawley rat. J Toxicol Pathol. **11**: 203–204. 1998. [CrossRef]
6. Sakashita S, Tsukada Y, Nakamura K, Tsuji I, and Hirai H. Experimental yolk-sac tumors produced by fetectomy without virus infection in rats. Int J Cancer. **20**: 83–86. 1977. [Medline] [CrossRef]
7. Van Hove L, Sobis H, and Vandeputte M. Viral-versus nonviral-induced yolk sac tumors in the rat. Oncodev Biol Med.

3: 97–109. 1982. [Medline]

8. Wewer U, Albrechtsen R, and Ruoslahti E. Laminin, a non-collagenous component of epithelial basement membranes synthesized by a rat yolk sac tumor. Cancer Res. **41**: 1518–1524. 1981. [Medline]

9. Damjanov I, and Sell S. Yolk sac carcinoma grown from rat egg cylinders. J Natl Cancer Inst. **58**: 1523–1525. 1977. [Medline]

10. Majeed SK, Alison RH, Boorman GA, and Gopinath C. Ovarian yolk sac carcinoma in mice. Vet Pathol. **23**: 776–778. 1986. [Medline]

11. Delacourt MC, Sobis H, and Vandeputte M. Immunofluorescent localization of alpha fetoprotein in yolk sac carcinomas of the rat. J Natl Cancer Inst. **57**: 1375–1377. 1976. [Medline]

12. Pirak M, Waner T, Abramovici A, Scolnik M, and Nyska A. Histologic and immunohistochemical study of a spontaneous choriocarcinoma in a male Sprague Dawley rat. Vet Pathol. **28**: 93–95. 1991. [Medline] [CrossRef]

13. Kajikawa S, Takeuchi A, Shirakai K, Nakano K, Izumisawa N, and Oishi Y. Spontaneous yolk sac carcinoma metastasizing to the heart and lung in a 10-week-old female Sprague-Dawley rat. 22nd JSTP Meeting abstract. 2006.

14. Arora S, Patel H, Mir P, Mishra S, Kalra A, Sawhney H, Duffoo F, Macera M, Abdu A, and Thelmo W. A bilateral primary yolk sac tumor of the lung associated with chromosome 3 polysomy: understanding the Oct 3/4 and Sox 2 interaction. Cancer Biol Ther. **14**: 1–4. 2013. [Medline] [CrossRef]

15. Inoue H, Iwasaki M, Ogawa J, Shohtsu A, Abe Y, Satoh S, and Osamura Y. Pure yolk-sac tumor of the lung. Thorac Cardiovasc Surg. **41**: 249–251. 1993. [Medline] [CrossRef]

16. Pelosi G, Petrella F, Sandri MT, Spaggiari L, Galetta D, and Viale G. A primary pure yolk sac tumor of the lung exhibiting CDX-2 immunoreactivity and increased serum levels of alkaline phosphatase intestinal isoenzyme. Int J Surg Pathol. **14**: 247–251. 2006. [Medline] [CrossRef]

17. Abdel Rahman AR, Ebied EN, Nouh MA, Gal AA, and Mansour KA. Primary yolk sac tumor of the lung. Ann Thorac Surg. **87**: 1925–1926. 2009. [Medline] [CrossRef]

18. Basoglu A, Sengul AT, Buyukkarabacak YB, Yetim TD, and Yildiz L. Pure yolk-sac tumor of the lung. Asian Cardiovasc Thorac Ann. **16**: 410–411. 2008. [Medline] [CrossRef]

19. Pont J, Pridun N, Vesely N, Kienzer HR, Pont E, and Spital FJ. Extragonadal malignant germ cell tumor of the lung. J Thorac Cardiovasc Surg. **107**: 311–312. 1994. [Medline]