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Case report

SPONTANEOUS LYMPHOMA IN A SMP30 KNOCK-OUT C57BL/6 MOUSE

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70-weeks-old female C57BL6 senescence marker protein 30 knock out mice exhibited anorexia, lethargy and enlarged abdomen because of an intraperitoneal mass. On necropsy, the mouse revealed a large brown-whitish mass located on the mesentery. The mass also exhibited systemic metastasis and spread over in various organs. On microscopic findings, the neoplastic masses were mainly composed of neoplastic round cells characterized by severe anisokaryosis, narrow cytoplasm, round nuclei, prominent nucleoli, and numerous mitotic figures (13-15 in a 400X field). Consequently, the present case was diagnosed as a metastatic lymphoma arising from a mesenteric lymph node, the tumor spread to other organs such as the intestine, kidney and thoracic cavity. According to previous studies, SMP30 plays an important role in inhibiting cancer in both human and mouse. Taken together, it seems that the present case can be used as a valuable asset for evaluating the potential risks of SMP30 depletion in developing lymphoma.

Keywords: experimental animal; lymphoma; mouse; SMP30; cancer

INTRODUCTION

Lymphoma is a tumor of the lymphatic system originating from various lymphocytes such as the T cell and B cell lines, and these types of tumors were categorized by various subtypes according to the 2008 WHO classification [1]. In humans, lymphoma accounts for 3.5% of all tumor cases and 2.84% of cancer death worldwide in 2020 [2]. According to previous studies, lymphoma is a malignant hematopoietic tumor occurring in companion animals such as dogs (13-114 per 100,000 dogs) and cats (total of 271 cases from 562,446 cats) [3,4]. Moreover, it is also frequently observed in laboratory animals including rodents (10-50% of aging mice) [5].

Lymphoma has many leading causes such as aging, infectious diseases, carcinogens, lifestyle diseases including obesity and smoking and autoimmune diseases [6-8]. Although there have been many developments in the field of classification, tools and

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analysis technique, the accurate pathogenesis and treatment of lymphoma are still challenging because it needs to consider various factors such as: immunophenotyping, cytogenetics, radiology, clinical data, microscopic examination including histology and cytology [7,9]. Thus, it seems that additional research on lymphoma is urgently needed. The senescence marker protein 30 (SMP30) is the first identified as an age-associated protein due to androgen-independently decreased patterns in aged rat livers, and it has a well-preserved protein structure across many vertebrate animal species [10]. SMP30 is distributed in various parenchymal organs such as the liver and kidney and performs a role in vitamin C biosynthesis except for primates and guinea pigs [11]. According to previous studies, SMP30 depleted animals exhibited increased susceptibility to harmful responses such as apoptosis and hypoxia mediated reactions [12]. SMP30 also plays an important role in maintaining calcium homeostasis by regulating the Ca^{2+} pump in various organelles including the plasma membrane, microsomes and mitochondria [13]. Interestingly, several previous studies also reported that the upregulation of intracellular Ca^{2+} seemed to mediate a cell death through receptor over activation [14]. Moreover, the previous studies also reported that the imbalance of calcium homeostasis is closely related with initiation of malignant tumors [15]. When considering that these inflammatory responses, cell death and calcium imbalance mediated by aging-related SMP30 depletion can induce tumor development, it is suggested that SMP30 depletion can be a high-risk factor for neoplastic disease including lymphoma. Similarly, many previous researches also suggested that the SMP30 plays an anti-cancer effect in various tumors such as hepatocellular carcinoma [16]. Therefore, it seemed that the loss of SMP30 is a high-risk factor for tumor occurrence. Here, we report a case of lymphoma arising from SMP30 KO mouse.

CASE PRESENTATION

The 70-weeks-old SMP30 KO female mouse was housed in a controlled environment (22±3°C; 30±10% relative humidity on a 12-hr light-dark cycle) with ad libitum access to food and vitamin C containing filtered tap water (vitamin C 1.5g/L). Suddenly, the animal exhibited anorexia, lethargy, and notable swelling of the abdomen due to the significantly enlarged intraperitoneal mass. Eventually, the mouse was euthanized, and necropsy was performed. At necropsy, the animal exhibited a firm, large brown-whitish mass in the abdominal cavity (Figure 1 A). The mass spread over to other organs including the kidney, thoracic cavity, and intestines (Figure 1 B-D). Briefly, the tissue samples were immediately fixed in 10% neutral buffered formalin and routinely processed. The paraffine sections (5 μm thick) were stained with hematoxylin and eosin (H&E). In the microscopic findings, the abdominal mass was generally composed of poorly differentiated round cells characterized by severe anisokaryosis, prominent nucleoli, high cellularity, coarse chromatin, strong invasiveness to adjacent tissue or blood vessels and numerous mitotic figures (13-15 in a 400X field) suggesting severe malignancy (Figure 2 A-C). Consistently, the neoplastic masses located on the kidney,

thoracic cavity and intestine also exhibited similar histopathologic characteristics to the abdominal mass (Figure 2 D and E). Following immunohistochemistry, analysis using Histostain® Plus Broad-Spectrum Kit (Cat#859043, Life Technologies, Carlsbad, CA, USA) revealed complete loss of SMP30 (COSMOBIO CO., LTD., SML-RO1001-EX, 1:500, Tokyo, Japan) in SMP30 KO mouse (Figure 3 A and B). Additionally, the neoplastic round cells exhibited strong positive responses for CD3 (Agilent, Santa Clara, A0452, 1:200, Santa Clara, USA) which is a major marker of T-lymphocytes (Figure 3 C and D) [17]. The differential diagnosis of the present case included mast cell tumor, histiocytic sarcoma, and plasma cell tumor. However, mast cell tumor was ruled out because the neoplastic round cells exhibited no cytoplasmic granules and completely negative responses for toluidine blue stain. Histiocytic sarcoma and plasma cell tumor were excluded because the neoplastic round cells exhibited centrally-located round nuclei and lack of cytoplasm. Therefore, the present case was diagnosed as a T-cell lymphoma spread to various organs such as the intestine, kidney and thoracic cavity in SMP30 KO mouse.

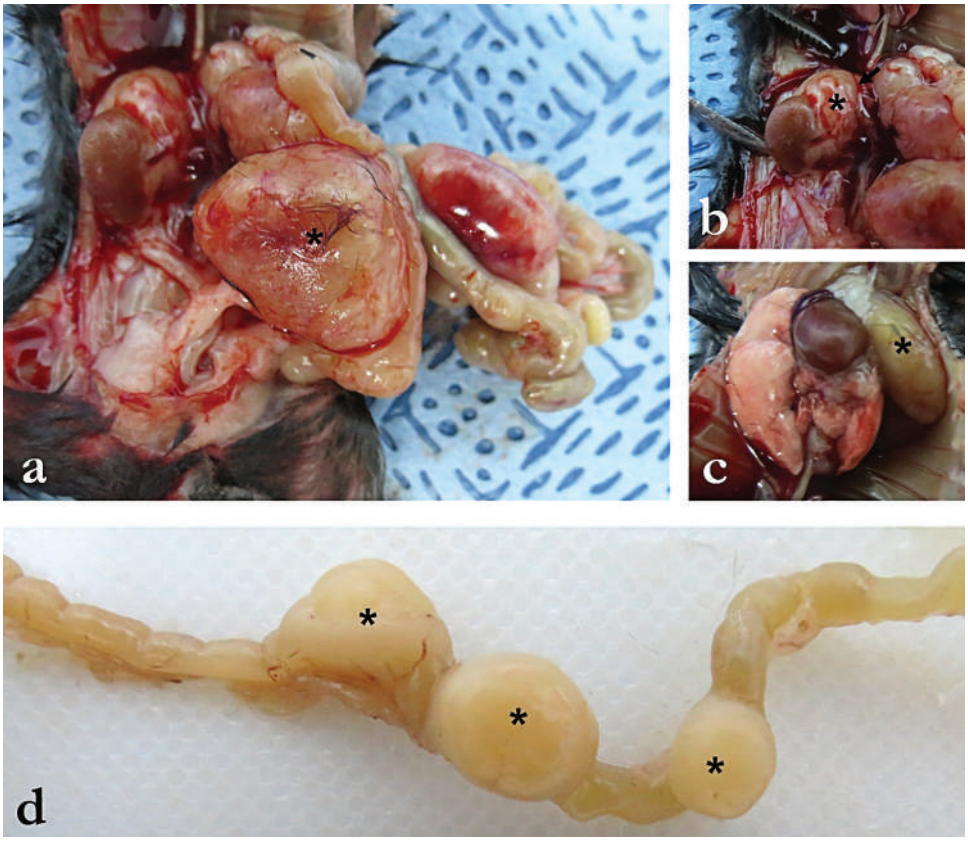

Figure 1. Neoplastic masses of a 70-week-old SMP30 KO mouse. Note the large abdominal masses in the peritoneal and thoracic cavity. **(A)**: The animal exhibited a large brownish-white mass around the mesentery (asterisk). **(B-D)**: Those neoplastic masses (asterisks) were observed with systemic metastasis.

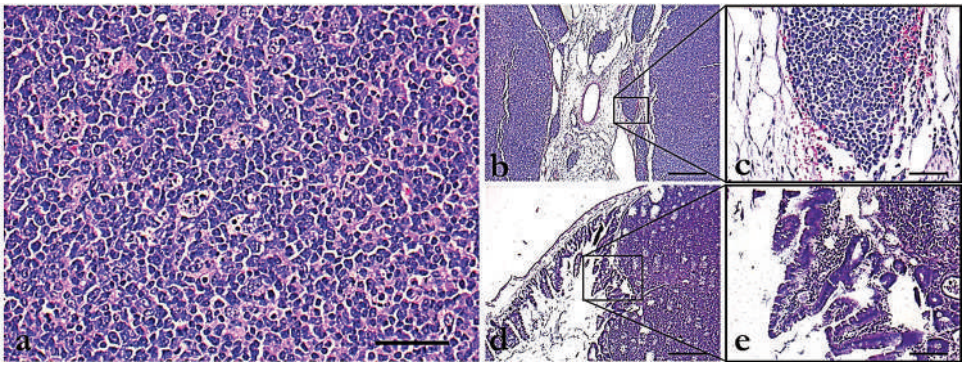

Figure 2. Representative image of neoplastic masses (Hematoxylin & Eosin; HE). **(A):** picture of abdominal mass. The mass was mainly composed of neoplastic round cells characterized by severe anisokaryosis and numerous mitotic figure (13-15X per 400X field). HE, Bar = 50μm. **(B and C)**: Picture of blood vessels in the neoplastic masses. The neoplastic cells exhibited severe invasiveness suggesting malignancy and metastasis of the tumor. HE, Bar = 400μm and 50μm. **(D and E)**: picture of the intestine and neoplastic masses. The Peyer's patches were almost completely replaced by neoplastic round cells. Note the narrowed lumen and infiltrations of neoplastic cells in the villi. HE, Bar = 100μm and 50μm.

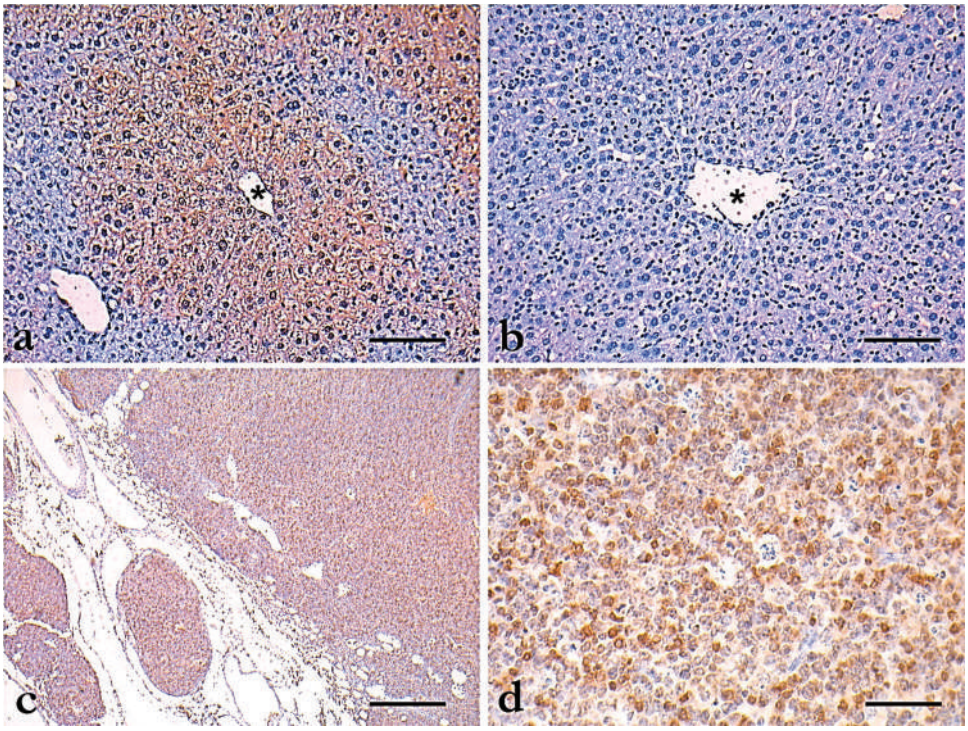

Figure 3. Immunohistochemistry for SMP30 expression in liver. **(A)**: Liver tissue of wild-type mouse revealed strong positive responses for SMP30 around a central vein area (Asterisk). Bar = 100 μm. **(B)**: Liver tissue of SMP KO mouse exhibited complete negative responses for SMP30. Bar = 100 μm. Immunohistochemistry for CD3 expression in neoplastic mass. **(C and D)**: The neoplastic round cells exhibited strong positive responses for CD3 suggesting lymphocytic origin. Bar = 400 μm and 50 μm.

Lymphoma is defined as a tumor arising from lymphocytes and is responsible 630,000 new tumor cases and 283,169 cancer deaths worldwide in 2020 [2]. Notably, the prevalence of lymphoma is nearly 2-fold higher in developed countries than in developing countries because of high proportions of older persons in these countries [2,18]. When considering that accelerated aging is one of the biggest problems worldwide, it seems that the clinical importance of lymphoma is also constantly increasing [19]. SMP30 is generally known as a major marker protein of senescence because of androgen-independent decreasing patterns with aging rat liver [10]. Therefore, the SMP30 KO mice is regarded as a mouse experimental aging model [20]. When considering that the depletion of SMP30 is mainly caused by aging, it seems that the analysis of the lymphoma in SMP30 KO mice will allow a better understanding of the mechanisms about lymphomagenesis. Previous research reported that the downregulation of SMP30 mediates various harmful lesions such as inflammatory responses and increased cell death [21]. According to the previous studies, the sustained inflammatory response is a predisposing factor for cancer [22]. SMP30 can also intermediate Ca^{2+} efflux in cells by activating the Ca^{2+} pump in the plasma membrane, and SMP30 depletion induces upregulation of intracellular Ca^{2+} concentration in living cells [23]. Previous investigation reported that these increased intracellular Ca^{2+} concentrations not only induce increased cell death but also provoke cancer development due to upregulated cell division [14,24,25]. Therefore, it seems that the loss of SMP30 can be a high-risk factor for cancer development via both sustained cell injuries and increased proliferations of tumor cells. According to a previous study, the amino acid sequence of SMP30 is highly preserved in many vertebrates including humans [10]. Therefore, it is also suggested that the loss of SMP30 is a potential risk factor of lymphoma in many other animal species. Moreover, recent studies reported that the newly revised WHO classification systems for haemopoietic tumors used in veterinary medicine are very comparable to those in human lymphoma classification [26]. Therefore, we believe that investigation of the animal lymphoma case could be important for understating pathogenesis of human lymphoma. Taken together, this study is the first report about the relationship between loss of SMP30 and lymphoma. We assumed that the present case can be used as a valuable data for understating the pathogenesis of lymphoma.

Authors' contributions

SWL carried out the autopsy, microscopic examination, and immunohistochemistry, and drafted the manuscript. SMB and YJL carried out the sample staining and contributed to data interpretation. JKP conceived of the study, participated in its design and provided final approval of the version to be published.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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SPONTANI LIMFOM KOD SMP30 NOKAUT C57BL/6 MIŠA

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Sedamdeset nedelja stara ženka C57BL6 marker protein 30 nokaut miša ispoljavala je znake anoreksije, letargije i uvećanja abdomena usled prisustava intraperitonealne mase. Prilikom obdukcije, na mezenterijumu uočeno je prisustvo velike braon-beličaste mase uz istovremeno prisustvo sistemskih metastaza u brojnim organima. Na mikroskopskim nalazima, neoplastične mase su uglavnom bile sastavljene od neoplastičnih okruglih ćelija koje karakteriše veoma izražena anizokarioza, redukovana citoplazma, okrugla jedra, istaknuta jedarca i brojne mitotske figure (13-15 u polju od 400x). Shodno tome, u ovom slučaju je dijagnostikovan metastatski limfom koji nastaje iz mezenteričnog limfnog čvora, tumor se proširio na druge organe kao što su creva, bubrezi i torakalna šupljina. Prema prethodnim studijama, SMP30 igra važnu ulogu u inhibiciji kancera i kod ljudi i kod miša. Na osnovu rezultata ispitivanja čini se da se ovaj slučaj može koristiti kao dragoceno sredstvo za procenu potencijalnih rizika SMP30 deplecije u razvoju limfoma.