The role of the lymphatic system in rabbit models for cancer metastasis research: a perspective from comparative anatomy

By

Hisashi OSHIRO

Department of Anatomic Pathology, Tokyo Medical University, Tokyo, Japan

– Received for Publication, July 22, 2014 –

Key Words: humans, lymphatic metastasis, mice, rabbits, rats

Summary: The elucidation of the pathogenesis of human diseases requires increasingly relevant and rigorous animal models. Therefore, investigators must select an appropriate mammalian model. Mice and rats are indispensable in the understanding of the mechanisms of human diseases, but other non-rodent mammals are required in certain situations. The rabbit is one such species. The rabbit exhibits greater biological similarities to humans than the mouse or rat, and the rabbit VX2 allograft cancer model has been used in a broad range of oncological studies, such as stromal responses, metastatic behaviors and therapeutic effects. Cancer cells in this model proliferate in a host rabbit that maintains a natural immunity, which makes this model attractive and unique. However, these examples constitute only a small number of advantages of a rabbit model. Numerous reports suggest that the rabbit is an attractive cancer-bearing animal model for the study of cancer metastasis and the lymphatic system. I briefly review the relevant medical literature and compare the rabbit lymphatic system with mice, rats and humans.

Introduction

The elucidation of the pathogenesis of human diseases requires increasingly relevant and rigorous animal models. Therefore, investigators must select an appropriate mammalian model. Mice and rats are indispensable in the understanding of the mechanisms of human diseases, but other non-rodent mammals are required in certain situations. The rabbit is one of these species. The rabbit exhibits greater biological similarities to humans than the mouse or rat (1), and the rabbit VX2 allograft cancer model has been used in a broad range of oncological studies, such as stromal responses, metastatic behaviors and therapeutic effects (2-9).

VX2 cancer cells in this model proliferate in a host rabbit that maintains a natural immunity, which makes this model attractive and unique (10). Furthermore, this model replicates human peritoneal carcinomatosis well (11). However, these examples may represent only a small number of advantages of a rabbit model. The rabbit may be an attractive cancer-bearing animal model for the study of cancer metastasis and the lymph flow. I briefly review the relevant medical literature and compare the rabbit lymphatic system with mice, rats and humans.

Comparative Anatomy of the Lymphatic Systems of Mice, Rats and Humans

Clinically, lymph flow is an important factor in the order of cancer metastasis, secondary lymphedema, and the mechanisms of malignant fluid accumulation in the serosal cavity. However, the lymph nodes and lymph trunks in humans do not exist in all mammals, and vice versa.

As a general rule, lower mammals have a smaller variety and number of lymph nodes (12). Spira investigated the utility of lymphocenters between different species in terms of lymph node development (13). Kawashima et al noted that mice have only 13 lympho-
centers that do not contain parotid, retropharyngeal, superficial cervical, ventral thoracic, dorsal thoracic or superficial inguinal lymphocenters (14). Notably, 100% (15/15) of NIH-strain mice lack bronchial lymph nodes, 73.1% (19/26) of DD-strain mice lack gastric lymph nodes, and 65.4% (17/26) of DD-strain mice lack bronchial lymph nodes (14). In contrast, rats have approximately 15 lymphocenters (15), which is equal to rabbits (16). Interestingly, the cranial ventral mediastinal lymph nodes of rats do not accept lymph flow from the lung, but these nodes do accept lymph flow from the stomach and the liver (15).

Another difference can be recognized using the classical concept of Kutsuna (17, 18), who defined the lymphatic trunk as a well-developed, single or main collecting lymphatic vessel originating from the efferent vessels of the last lymph nodes and pouring directly into the thoracic duct or vein without relaying lymph nodes. In other words, mice (14), rabbits (16) and humans (17, 18) possess an intestinal lymphatic trunk but rats do not (15). These unique features distinguish rats from mice, rabbits and humans.

Notably, rabbits have buccal (facial), submental, preauricular, retroauricular, pretracheal, paratracheal, posttracheal, intercostal and gastric cardiac lymph nodes, which are also observed in humans but do not normally exist in mice and rats (14-16). These anatomical differences between species influence the order of lymph node metastasis, which is important in sentinel lymph node navigation surgery. A cancer-bearing rabbit model is a good model for the training of sentinel lymph node biopsy of gastric cancer (19).

The lymphatic drainage route from the lung in rabbits is more similar to humans than mice or rats. The contributory nodes in rabbits consist largely of four node types (cranial ventral mediastinal group I, cranial ventral mediastinal group II, paratracheal and tracheobronchial nodes) (20), whereas mice have two types of nodes (mediastinal and bronchial nodes) (14), and rats have only one type of node (cranial tracheobronchial node) (15). Moreover, rabbits, unlike mice or rats, often possess bronchopulmonary, caudal tracheobronchial, prebronchial, parabronchial and posttracheal nodes, which contribute to the lymphatic drainage from the lung similarly to humans (20).

Direct communications between the intrathoracic lymphatic vessels and the cervical lymph nodes are demonstrable in rabbits, with 44% on the right side and 24% on the left side (21). These frequencies are almost equivalent to humans, who exhibit 48% on the right side and 21% on the left side (22). Furthermore, rabbits demonstrate three patterns of lymphatic drainage from the lung: ipsilateral, crossing and indirect pathways (20), which are also found in humans (23). This feature suggests that rabbits, unlike mice or rats, are an appropriate model for the study of skipped, contralateral mediastinal or supraclavicular lymph metastases (24-28).

Similar to humans, rabbits also possess superficial and deep lymphatic drainage systems in the upper extremities (15, 18), whereas this dual pathway is not well constructed in mice and rats (14, 15). This feature makes rabbits a more appropriate model than mice or rats for secondary lymphedema in the upper extremities.

**Serosal Membranes, Lymphatic Stomata and Coelomic Fluid Drainage Routes**

Rabbits are also a more suitable peritoneal dialysis model than mice or rats because rabbits are capable of long-term peritoneal dialysis, and rabbits exhibit a similar ratio of peritoneal surface area and exchange volume (29). This similarity implies a certain resemblance of the functional anatomy of the serosal and lymphatic systems between rabbits and humans. The following factors may influence fluid turnover in the serosal cavity of mammals: [1] Starling’s hypothesis; [2] Fick’s law of diffusion; [3] the serosal pressure associated with organ movement and posture; [4] active transport by mesothelial cells; and [5] a lymphatic drainage system via lymphatic stomata (30, 31). Of these factors, the lymphatic drainage system via lymphatic stomata likely plays an important role (30–34), which may contribute up to approximately 75–80% of the fluid absorption rate in the abdominal and pleural cavities (33, 35).

Anatomical differences in the lymphatic vessels may affect the outcomes of lymphogenous metastases due to lymphatic drainage routes via the lymphatic stomata from the serosal cavity (36). The conduit lymphatic vessels drain into several areas in the human adult diaphragmatic pleura: [1] towards the lower sternal portion; [2] to the boundary adjoining the intercostal pleura; [3] to the junction of the vertebrae and ribs; [4] to the aortic hiatus; and [5] to the esophageal hiatus (37). Furthermore, the mediastinal pleura drains into the following regions: [1] the upper and lower portions of the sternum; [2] the reflection region of the pericardium; [3] the hilum of the lung; [4] the aortic hiatus; and [5] the esophageal hiatus (37).

When VX2 cancer cells are injected into the peritoneal cavity of a healthy rabbit, these cells enter into diaphragmatic lymphatic vessels via the lymphatic stomata on the diaphragmatic peritoneum and cause local lymphatic obstruction (38). This rabbit model may elucidate the mechanisms of cancer dissemination via lymphatic stomata and the accumulation of ascites or pleural effusion, as investigated in human cases (39–42).

Recent studies have demonstrated that the thoracic duct plays a specific role in lymphatic drainage from the serosal cavity of quadrupeds (43–45). Therefore, anatomical differences in the thoracic duct and the duct tributaries may also affect the outcomes of lymphogenous metastases. According to the anatomical classification of
the thoracic duct of Adachi (46), rabbits possess two duct types (types IV and VI) (16) that overlap with several human duct types (types III, IV, V, VI and IX) (47). Mice and rats possess only one type (type VI), in which the thoracic duct runs along the right side of the descending aorta and drains into the left venous angle, but the duct never splits the flow into the right venous angle (12).

Conclusion

The reasons described above clearly demonstrate that, compared with the lymphatic system of mice and rats, the lymphatic system of rabbits more closely resembles that of humans. Therefore, the rabbit can be a useful cancer-bearing model for cancer metastasis research that is focused on the lymphatic system.

Author disclosure statement: No competing financial interests exist.

Acknowledgments

This research was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (No. 20790993 and No. 24590242), Exploratory Research Culture, Sports, Science and Technology of Japan (No. 26390993), Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (No. 20790993), and the Ministry of Education, Culture, Sports, Science and Technology of Japan (No. 20790993).

References

27) Anami K, Yamashita S, Yamamoto S, Chuo M, Tokuishi K, Moro T, Mori H, Kawahara K: Contralateral mediastinal lymph...


