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Original Article

Blood vessels in the mouse tail: progress in anatomical studies

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Abstract: Objective: To investigate the structural characteristics of caudal blood vessels in the vasculature of the mouse tail by multiple techniques. Methods: We investigated the vascular structure of the mouse tail using a range of techniques, including gross anatomical microscopy, dual-color micro-emulsion perfusion, Micro-computed tomography (micro-CT), micro-angiography, histopathology, X-ray microangiography, and scanning electron microscopy. In particular, we performed a comprehensive examination of the transverse caudal vessels and the deep caudal vascular system. Results: The vasculature of the mouse tail consists of two circulatory systems: the longitudinal circulation system and the local circulation system. The longitudinal system comprises three groups of blood vessels: the middle caudal vessels and the left and right caudal vessels. In addition, we proposed standardized nomenclature for these vessels. Conclusion: We identified two modes of blood circulation in the mouse tail: (1) longitudinal circulation running along the length of the tail and (2) local circulation. These blood vessels were categorized into three types: superficial vessels, deep vessels, and communicating vessels.

Keywords: Mouse anatomy, caudal blood vessels, transverse caudal artery, deep caudal artery, vascular imaging

Introduction

Laboratory mice are widely used in biomedical research, with the vasculature in the tail frequently serving as key sites for experimental procedures. In a previous study, Steel et al. demonstrated that injection into the lateral tail vein of mice is an effective route for drug delivery [1]. Similarly, Jin et al. confirmed that the injection of HepG2 cells into the tail vein of mice could be used to investigate liver metastasis [2]. Other researchers have demonstrated that tail vein injection is a valuable method for developing various *in vivo* models of liver disease [3]. Advances in emerging technologies, such as transmission electron microscopy (TEM) [4], and super-microsurgery, which demands exceptional hand-eye coordination, have further enhanced the anatomical investigation of laboratory mice [5]. However, our existing knowledge of the anatomical structure of mouse tail vessels is limited to the superficial vessels. To date, there have been no specific investigations into the specific vasculature of the mouse tail. In this study, we performed an in-depth investigation of the vasculature in the mouse tail using multiple advanced techniques, including gross anatomical microscopy, dual-color microemulsion perfusion, Micro-computed tomography (micro-CT), micro-angiography, histopathology, X-ray microangiography, and scanning electron microscopy. To our knowledge, this is the first comprehensive characterization of the deep caudal vascular system in the mouse. Our findings pave the way for future research and establish a solid foundation for further exploration in this field.

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Materials and methods

Gross anatomy and microscopic imaging

Ten male C57BL/6 mice, weighing 24-27 g, were acquired from Shanghai SLAC (Shanghai, China). The mice were housed at the barrier facility of Shanghai Laboratory Animal Research Center. All animal experiments were carried out in compliance with institutional guidelines and were approved by the Animal Care and Use Committee of Shanghai Laboratory Animal Research Center. Mice were euthanized by exposure to carbon dioxide (CO_2), and their tails were dissected and examined by surgical microscopy.

Colored silicone vascular perfusion and tissue clearing

For perfusion and clearing, we purchased 60% Armenian silicone (Shenzhen Jitian Chemical Co., Ltd.) and tissue fix solution (Wuhan Servicebio Technology Co., Ltd.). To prepare the silicone perfusion solution, we diluted 60% Armenian silicone with normal saline (1:1), and mixed this with red or blue dyes. Then, we prepared a tissue clearing solution by dissolving 10 g of KOH in 800 mL distilled water before adding 200 mL of glycerin and mixing thoroughly.

For silicone perfusion, mice were anesthetized by intraperitoneal injection with sodium pentobarbital (150 mg/kg). Once the mice were anesthetized, heparin sodium was injected into the sublingual vein (2000 units/mL, 0.5 mL). Next, we exposed the abdominal cavity, clamped the left and right common iliac artery and vein, intubated the abdominal aorta and perfused with normal saline. A 0.5 mm incision was created at the junction of the left renal vein and the posterior vena cava to create an outlet for the perfusion solution. The perfusion was stopped when the effluent became clear, and a cannula was inserted at the opening of the posterior vena cava and clamped caudally. Approximately 1 cm of the tail tip was amputated. Then, we perfused the red silicone buffer from the abdominal aorta and the blue silicone buffer from the posterior vena cava. The tip of the tail was then ligated and 100 μL of silica gel was infused until excess gel was seen to flow from the tip of the tail. At this point, a further 100 μL of silica gel was perfused and the tip of the tail was ligated. The tail was then cut at the proximal end of the ligation line and stored in ethanol at 4°C. After 24 hours, the tail was dissected and examined by microscopy.

For tissue clearing, silicone perfused specimens were sequentially immersed in anhydrous ethanol for 1 week, 1% KOH solution for 1 week, and tissue clearing solution for 1 week. Then, the specimens were dissected and photographed under a microscope.

Micro-CT scanning

ICR male mice, 8 weeks-of-age, were provided by Shanghai SLAC (Shanghai, China). The mice were housed at the barrier facility of Westlake University. All procedures in this study were carried out with the approval of the Institutional Animal Care and Use Committee of Westlake University.

To create a barium sulfate suspension, we purchased dry barium sulfate (type I) for suspension (Qingdao Hongdie New Material Co., Ltd., Chinese medicine H20163180). Next, 1% Evans blue was dissolved in normal saline, and barium sulfate was added to the solution at a concentration of 0.5 g/mL to generate a contrast agent. Mice were anaesthetized (isoflurane: 3% induction and 2% maintenance), the abdomen and hindlimbs were shaved, and the abdominal cavity was exposed. Next, the abdominal aorta was intubated caudally, and the barium sulfate suspension was perfused slowly. When the tip of the tail was stained blue, a further 50 μl of contrast solution was perfused; then, the perfusion was terminated. After 15 minutes, the animals were scanned with a Micro-CT scanner (Bruker SkyScan1276 Micro CT) with the following settings: 70 kV, 200 μA, 20.62 μm pixel size, 336 ms exposure, and a 0.5 mm Al filter. The scanning time was 14 min and 27 s. Data reconstruction was performed by NRecon.

Micro-angiography

Ten BALB/C female mice, weighing 26-28 g, were acquired from the Department of Laboratory Animal Science, Health Science Center, Peking University. The mice were housed at the barrier facility of Peking University. All procedures in this study were carried out with the approval of the Institutional Animal Care and Use Committee of Peking University.

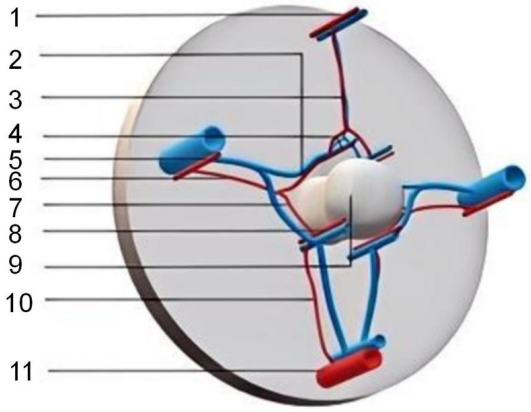

Figure 1. Three-dimensional (3D) schematic diagram of blood vessels in the mouse tail. Only the midline and left arteries are labeled, the veins and caudal cutaneous vessels are not shown. 1. Dorsal caudal artery; 2. Dorsal transverse caudal artery; 3. Distal dorsal transverse caudal artery; 4. Dorsal deep caudal artery; 5. Lateral caudal artery; 6. Communicating branch of lateral caudal artery; 7. Distal ventral transverse caudal artery; 8. Ventral deep caudal artery; 9. Caudal vertebrae; 10. Proximal ventral transverse caudal artery; 11. Middle caudal artery.

To generate a barium sulfate suspension contrast agent, we mixed 20 g of dry barium sulfate (type I) for suspension (Qingdao Hongdie New material Co., Ltd., Chinese medicine H20163180) with 50 mL of 1% Evans blue saline. For perfusion, mice were injected with sodium pentobarbital (50 mg/kg) intraperitoneally and sodium heparin (0.5 mL of 2000 units/mL) via the orbital venous sinus. Once anesthetized, the posterior vena cava was intubated caudally and ligated, then the barium sulfate contrast agent was perfused. When the tip of the tail became blue, the perfusion was stopped and the tail was examined by X-ray (UltraFocusDXA; Faxitron, Tucson, AZ, USA).

Electron microscopy

Three male and three female C57BL/6 mice, 8 weeks-of-age, were used for electron microscopy. The mice were housed at the barrier facility of Peking University. Cross-sectional electron microscopy scanning was then performed on mouse tails using a FEI Helios Nanolab G3 UC DualBeam FIB/SEM.

Pathology

Male C57BL/6 mice (24-27 g) were obtained from Shanghai SLAC (Shanghai, China). The mice were euthanized by CO_2 asphyxiation, and their tails were immediately excised and placed in formalin for one week to ensure proper fixation. Since the tails contained bone and cartilage, decalcification was performed by transferring the tissues to a decalcification solution and at room temperature for an appropriate period (typically 1-2 weeks). Following decalcification, the samples were processed through a graded ethanol series to remove water. Then, the samples were embedded in paraffin to facilitate sectioning with a microtome at a thickness of 4 μm. Next, the sections were stained in hematoxylin and eosin (HE) to enhance morphological visualization. Finally, the stained sections were examined under a light microscope to assess tissue morphology.

Results

Identification of two circulative blood routes in the mouse tail

We identified two types of circulative blood in the mouse tail involving both longitudinal and local systems. The longitudinal blood vessels included the middle and lateral caudal arteries and veins. The local blood vessels surrounded the caudal vertebrae, including the dorsal, transverse, deep, and cutaneous arteries and veins. In the deep and cutaneous blood vessels, the adjacent units lay opposite each other in the intervertebral space (**Figures 1**, **2**).

Sacral artery

The sacral artery is located in the second sacral vertebra. We found that the middle sacral artery branched out into a lateral sacral artery on each side. The lateral sacral artery proceeded back along the middle sacral artery, surpassed the middle sacral artery, reached the third caudal vertebrae, and branched out to the transverse sacral artery and transverse caudal artery at the middle level of each sacral vertebra and caudal vertebrae (**Figure 3**).

Middle caudal artery and collateral caudal artery

The middle caudal artery was visualized as a horizontal and backwards extension of the middle sacral artery at the posterior end of the sacrum and ran longitudinally to the tip of the tail in a subcutaneous position. The middle

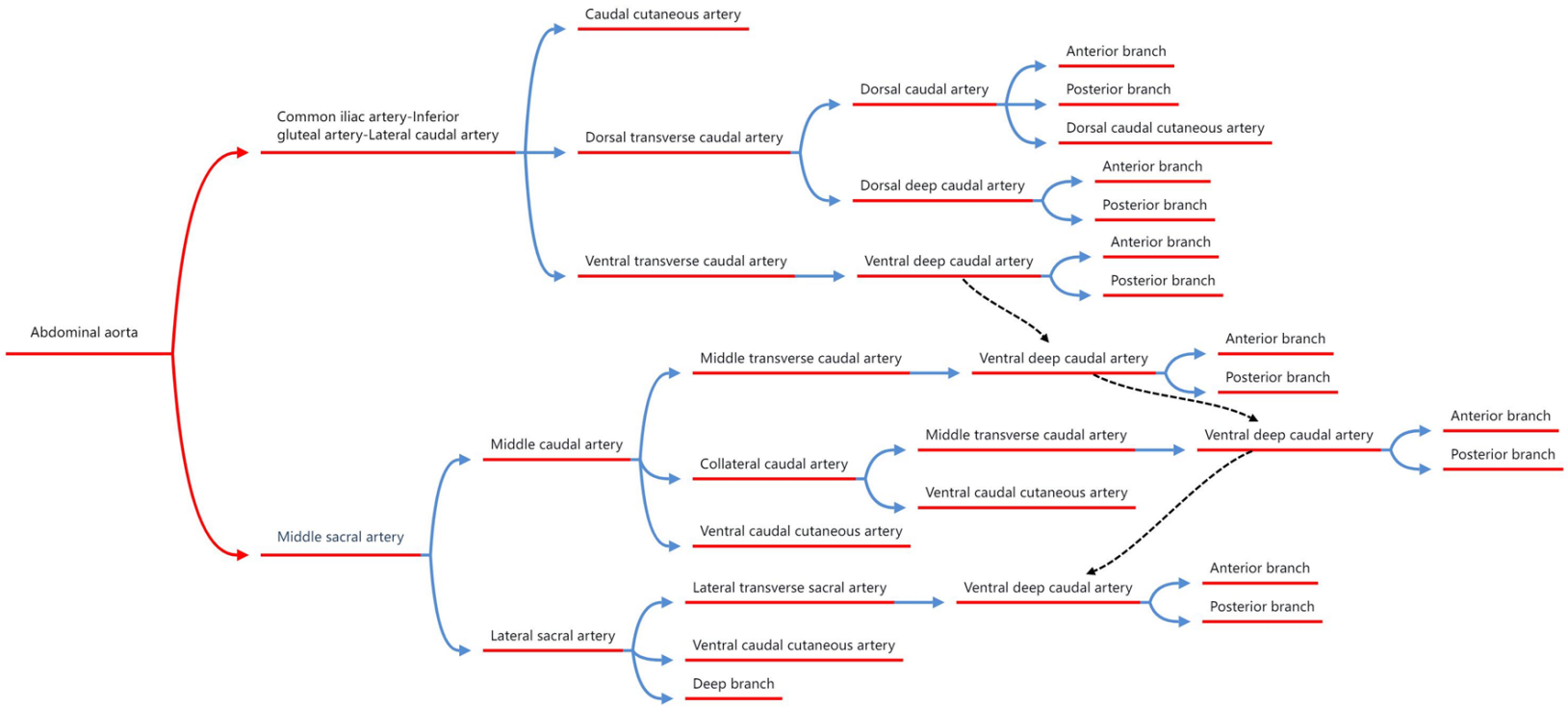

Figure 2. Schematic diagram of caudal blood vessels in mice.

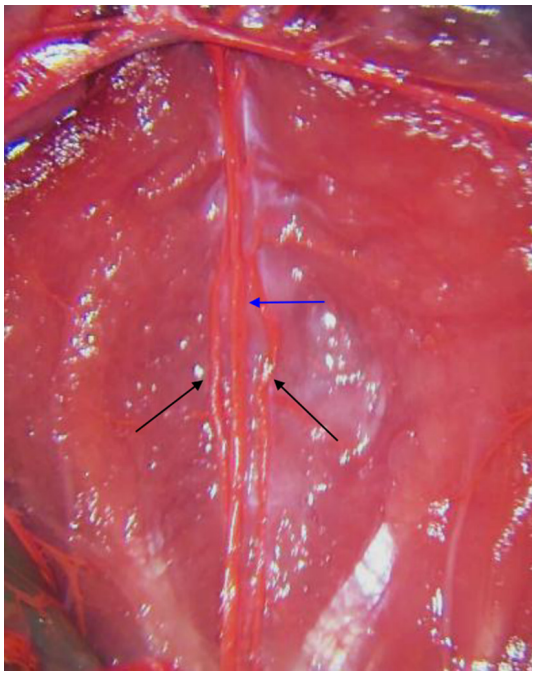

Figure 3. Anatomical diagram shows perfusion with arterial silicone. The black arrow indicates the lateral sacral artery while the blue arrow indicates the middle sacral artery.

caudal artery presents as a superficial vessel accompanied by the middle caudal vein (**Figure 4**). We identified several collateral caudal arteries at the third caudal vertebrae; these were arranged in a horizontal manner (**Figure 5**). After the ninth caudal vertebrae and in the middle of each caudal vertebrae, we observed a transverse caudal artery branching out to the left and right; this artery lay deep in caudal abdominal muscle (**Figure 6A**). The diameter of the middle caudal vein was significantly smaller than that of the middle caudal artery. The collateral caudal arteries were superficial vessels; we did not identify collateral caudal veins. Before and after the fifth caudal vertebrae, we identified two left and right branches from the middle caudal artery. These are divided into the anterior and posterior branches, each extending to the base and tip of the tail, located in the third to ninth caudal vertebrae (**Figure 6B**). Small branches provide blood supply to the root of the tail. The collateral caudal artery branched out from the middle transverse artery to the deep left and right in the middle of each caudal vertebrae surrounding its location (**Figure 6C**).

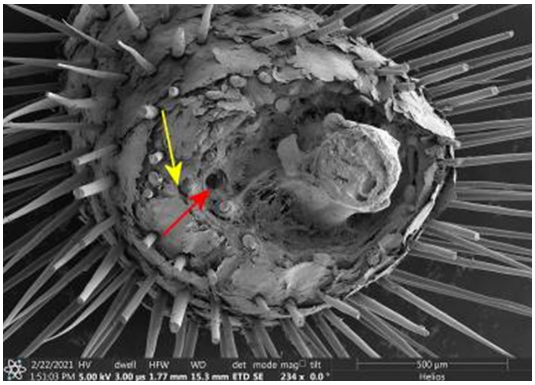

Figure 4. Electron microscopy image of a mouse tail. The red arrow indicates the middle caudal artery while the yellow arrow indicates the middle caudal vein. The vein is smaller than the artery.

Lateral caudal artery

The lateral caudal artery originated from the inferior gluteal artery and ran longitudinally through the entirety of the left and right tail in a subcutaneous manner (**Figure 7**). The lateral caudal artery branched out a communicating branch in the middle of each caudal vertebrae to communicate with the transverse caudal artery (**Figure 8**), while the superficial branches of the lateral caudal artery entered the skin to form the cutaneous arteries on both sides of the tail. We also identified lateral caudal veins accompanying the artery.

Transverse caudal artery

The transverse caudal artery is composed of distal and proximal segments of the lateral ventral transverse caudal artery and the lateral dorsal transverse caudal artery, forming a key part of the local blood circulatory in the tail. The transverse caudal artery originated from the collateral caudal or middle caudal artery and branched out one transverse abdominal artery to each side of the tail. The transverse caudal artery was found to be composed of four segments: (1) from the middle caudal artery or collateral caudal artery to the ventral deep caudal artery (the proximal segment of the lateral ventral transverse caudal artery); (2) from the ventral deep caudal artery to the communicating branch of the lateral caudal artery (the distal segment of the lateral ventral transverse caudal artery); (3) from the communicating branch of the lateral caudal artery to the dorsal deep

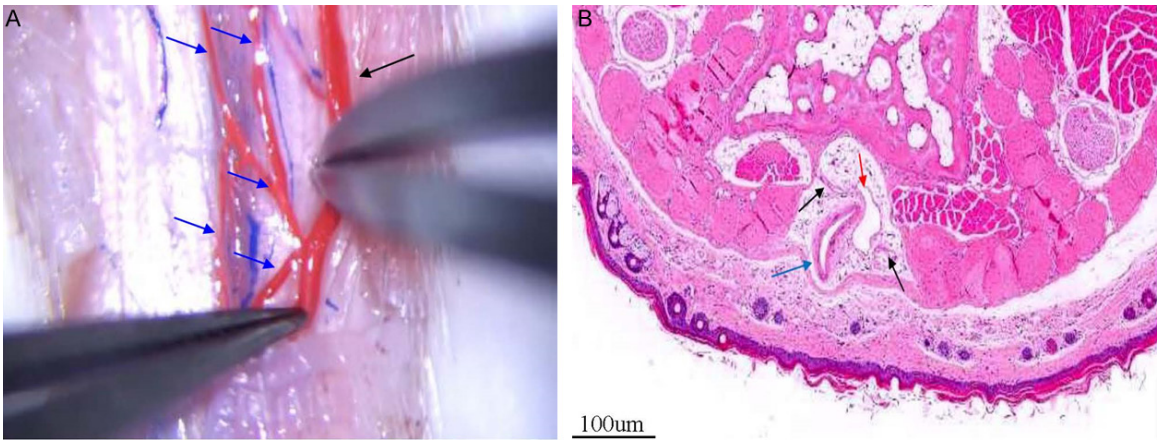

Figure 5. Pathological HE staining of the middle caudal artery and middle caudal vein. A: Several collateral caudal arteries branched out horizontally at the third caudal vertebrae. The black arrow indicates the middle caudal artery while the blue arrow indicates the collateral caudal artery. B: HE staining of a mouse tail. The black arrow indicates the collateral caudal artery while the blue arrow indicates the middle caudal artery; the red arrow indicates the middle caudal vein.

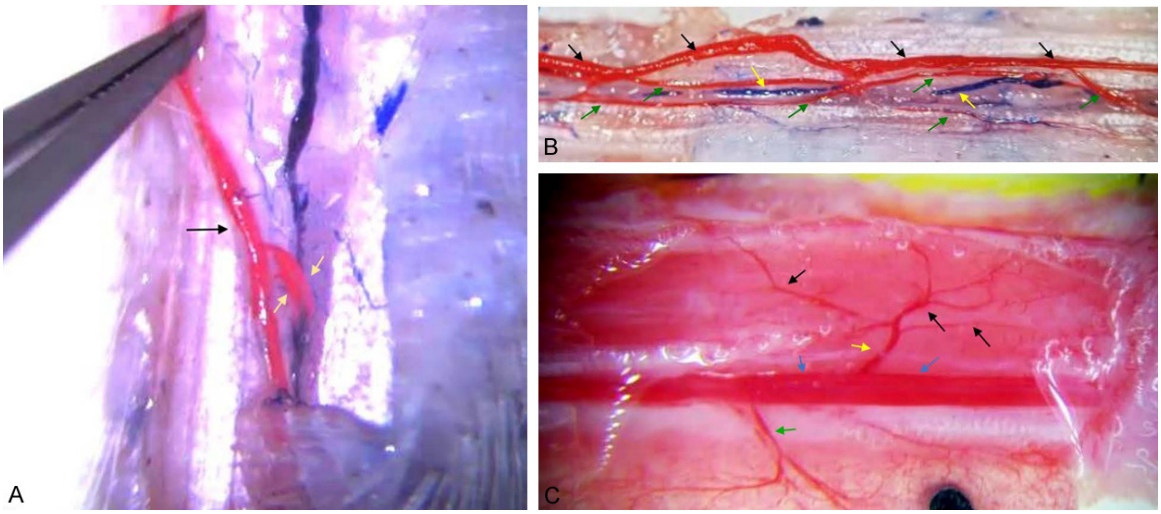

Figure 6. Pathological analysis of the median tail artery and the collateral caudal artery. A: The middle caudal artery branched out of the transverse caudal artery from the ninth caudal vertebrae. The black arrow indicates the median tail artery while the yellow arrow indicates the collateral caudal artery. B: Silicone perfusion dissection of the ventral caudal vessels. The black arrow indicates the middle caudal artery, the green arrow indicates the collateral caudal artery, and the yellow arrow indicates the middle caudal vein. C: Monochrome silicone perfusion dissection of the deep ventral caudal vessels. The green arrow indicates the tail ventral cutaneous artery, the blue arrow indicates the collateral caudal artery, the black arrow indicates the dorsal deep caudal artery, and the yellow arrow indicates the ventral transverse caudal artery.

caudal artery (the proximal segment of the lateral dorsal transverse caudal artery) and (4) from the dorsal deep caudal artery to the dorsal caudal artery (the distal segment of the lateral dorsal transverse caudal artery).

Deep caudal artery and deep caudal vein

The dorsal deep caudal artery provides local blood circulation surrounding each of the caudal vertebrae. There were four deep caudal arteries for each vertebra: the left ventral deep caudal artery, the left dorsal deep caudal artery, the right ventral deep caudal artery, and the right dorsal deep caudal artery. The deep abdominal artery originated from each transverse abdominal artery. The anterior and posterior branches of the caudal vertebrae were adjacent to each other and ran longitudinally

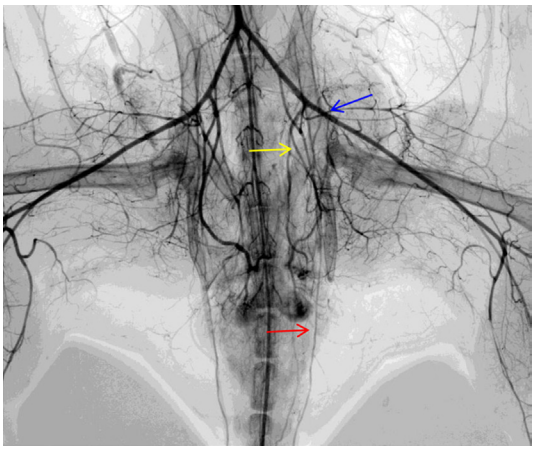

Figure 7. X-ray image of a mouse artery, the blue arrowhead indicates the common iliac artery, the yellow arrow indicates the inferior gluteal artery, and the red arrowhead indicates the lateral caudal artery.

through the entire tail. The deep dorsal artery was composed of the anterior and posterior branches of each segment of the transverse dorsal artery on the deep surface of the tail muscle (**Figure 9A**). The anterior and posterior branches of the caudal vertebrae lay adjacent to each other and ran longitudinally through the entire tail (**Figure 9B**), thus providing blood circulation for each caudal vertebra. Blood flowed from the beginning of the anterior and posterior branches in the middle of the caudal vertebrae to the intervertebral space in the tail. The dorsal deep caudal artery was accompanied by a dorsal deep caudal vein but were not in close proximity (**Figure 9C**); we also identified a venous valve in the vein (**Figure 9D**).

Dorsal caudal artery web and dorsal caudal vein

We identified anterior and posterior branches of the dorsal transverse caudal artery in the subcutaneous fascia when it reached the end of the tail. This artery interlaced with the posterior and anterior branches of the adjacent caudal vertebrae units to form a network of dorsal tail veins, thus providing local blood circulation for the caudal vertebrae. Blood flowed from the starting point of the middle anterior and posterior branches of the caudal vertebrae to the anterior and posterior tail intervertebral space (**Figure 10A**).

The dorsal caudal artery was accompanied by a dorsal caudal vein which was a single vein lying behind the ninth caudal vertebrae; this formed a chain with the dorsal vein in front of the ninth caudal vertebrae. The vein terminated at the junction of the third and fourth caudal vertebrae, and the communicating branches of the dorsal caudal vein were often connected with the lateral caudal vein (**Figures 10B**, **11**).

Caudal cutaneous artery

We identified four caudal cutaneous arteries for each vertebra in the tail: the ventral caudal cutaneous artery, the left cutaneous artery, the right cutaneous artery, and the dorsal caudal cutaneous artery. Four superficial vessels (**Figure 12**) branched out vertically to the skin and spread horizontally under the dermis. This formed the local blood circulation for the caudal vertebrae Caudal cutaneous arteries were accompanied by caudal cutaneous veins.

Vascular nomenclature

Given the fact that there are thousands of dorsal caudal vessels, we next generated specific nomenclature for these vessels to facilitate future research. The principle underlying our nomenclature was as follows: superior blood vessels + left/right/ventral/dorsal. The vein of the same name is not repeated here.

The middle caudal artery (MCA); one branch, belonging to the longitudinal blood vessels.

Lateral caudal artery (LCA); one on the left and one on the right, belonging to the longitudinal blood vessels.

The dorsal caudal artery (DCA) belongs to local circulatory vessels. Each local dorsal caudal artery was given its own name (for example, the fifth caudal vertebrae artery should be referred to as the ‘dorsal caudal artery-5 DCA5’).

Transverse caudal artery (TCA); each caudal vertebrae unit was found to have four transverse caudal arteries, as given below: (1) Left lateral ventral transverse caudal artery (LLVTCA). (2) Right lateral ventral transverse caudal artery (RLVTCA). (3) Left lateral dorsal transverse caudal artery (LLDTCA). (4) Right lateral dorsal transverse caudal artery (RLDTCA). (5) Each local transverse artery was given its own name. For example, the left middle transverse artery of the fifth caudal vertebrae should

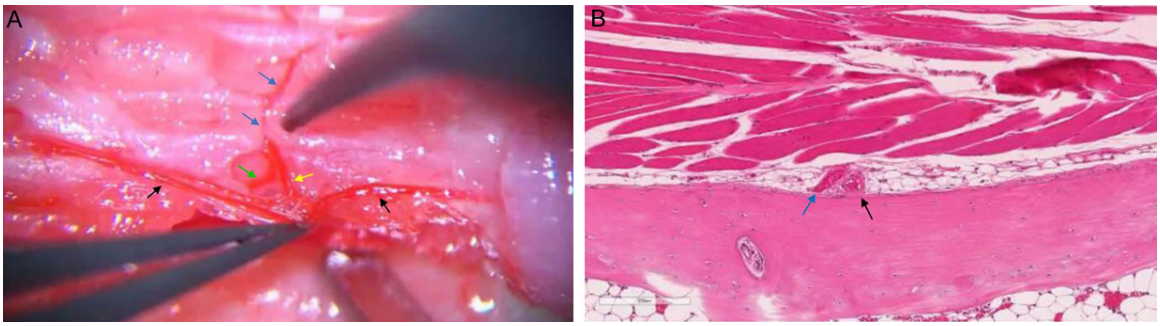

Figure 8. Pathological analysis of the transverse caudal artery and the transverse caudal vein. A: Monochromatic silicone perfusion dissection of the collateral caudal vessels. The green arrowhead indicates the lateral ventral transverse caudal artery, the blue arrow indicates the lateral dorsal transverse caudal artery, the black arrow indicates the lateral caudal artery, and the yellow arrowhead indicates the communicating branch of the lateral caudal artery. B: HE staining of pathological sections of a mouse tail. The black arrow indicates the transverse caudal artery, and the blue arrow indicates the transverse caudal vein.

Figure 9. Pathological and HE analysis of the dorsal deep caudal artery. A: Dorsal deep caudal artery (caudal vertebrae unit). The black arrowhead indicates the lateral caudal artery, the green arrow indicates the dorsal deep caudal artery, and the yellow arrow indicates the dorsal transverse caudal artery. B: The dorsal deep caudal vein, the black circle indicates the dorsal deep caudal vein, and the black arrowhead indicates the lateral caudal vein. C: The anterior and posterior branches of the dorsal deep caudal artery lay adjacent to each other. D: HE staining of a pathological section acquired from a mouse tail. The black arrow indicates the dorsal deep caudal vein valve.

be referred to as the 'left lateral ventral transverse caudal artery-5, LLVTCA5'.

Deep caudal artery (DeCA); we identified four deep caudal arteries, as given below: (1) Left ventral deep caudal artery (LVDeCA). (2) Right ventral deep caudal artery (RVDeCA). (3) Left dorsal deep caudal artery (LDDeCA). (4) Right dorsal deep caudal artery (RDDeCA). (5) Examples of deep caudal arteries: 1. The left deep caudal artery: 'left deep ventral caudal artery-5, LVDeCA-5' at the 5th caudal vertebrae. 2. Anterior branch of the left ventral deep artery-5 (LVDA5AB): the left anterior branch of the deep abdominal and tail artery at the 5th caudal vertebrae. 3. Posterior branch of the left ventral deep artery-5, LVDA5PB): the posterior branch of the left deep abdominal and tail artery at the 5th caudal vertebrae.

Caudal cutaneous artery: we identified four caudal cutaneous arteries in each tail unit, as

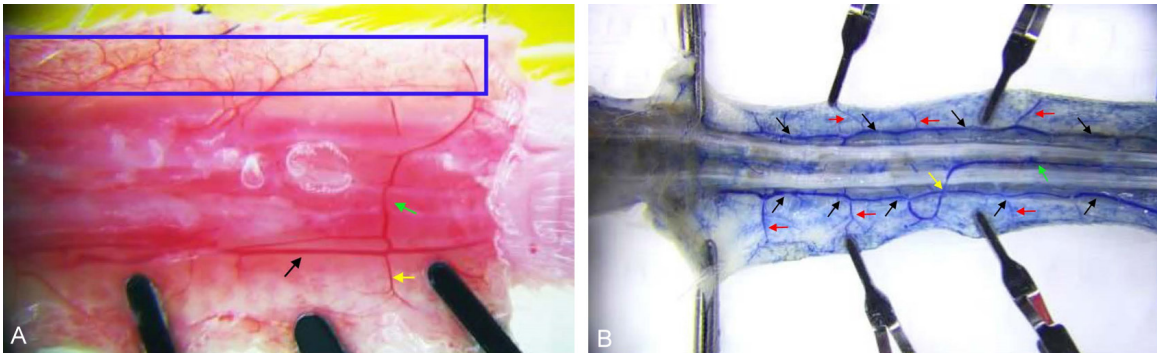

Figure 10. Pathological results of the dorsal caudal artery web. A: Dorsal caudal artery web. The blue frame indicates the dorsal caudal artery web, the black arrow indicates the lateral caudal artery, the green arrow indicates the transverse caudal artery, and the yellow arrow indicates the Caudal cutaneous artery. B: The communicating branch of the dorsal caudal vein and the terminal point of the dorsal caudal vein. The red circle indicates the end of the dorsal caudal vein, the black arrow indicates the lateral caudal vein, the green arrow indicates the dorsal caudal vein, the yellow arrow indicates the communicating branch of the dorsal caudal vein, and the red arrow indicates the caudal cutaneous vein.

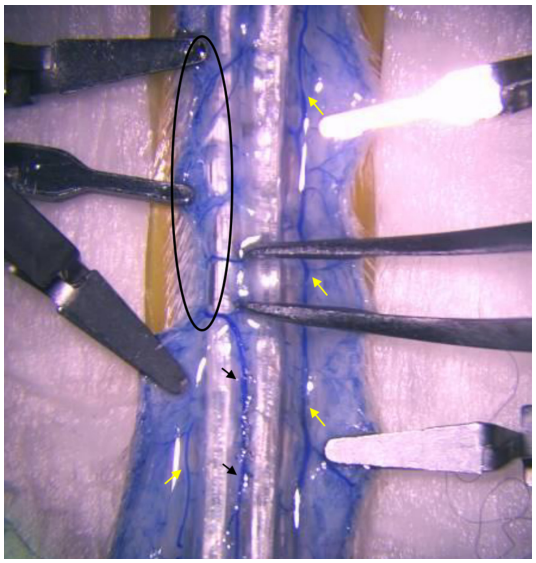

Figure 11. Monochromatic silicone perfusion anatomy of the dorsal caudal vein. The surgical tweezers indicate the ninth caudal vertebrae. The black circle indicates the dorsal caudal vein chain, the black arrowhead indicates the dorsal caudal vein behind the ninth caudal vertebrae, and the yellow arrowhead indicates the lateral caudal vein.

given below: (1) Ventral Caudal cutaneous artery (VCCA). (2) Left Caudal cutaneous artery (LCCA). (3) Right Caudal cutaneous artery (RCCA). (4) Dorsal Caudal cutaneous artery (DCCA). (5) Example of a specific caudal cutaneous artery: the ventral caudal artery of the 5th caudal vertebrae should be referred to as the 'ventral caudal artery-5, VCCA5'.

A comparison table, referred to as **Table 1**, has been added for clarity.

Discussion

Given the widespread use of the mouse tail in scientific research, there is a critical need to gain an enhanced understanding of the vascular anatomy of the mouse tail. Over recent years, the advancement of super-microsurgery has enabled precise micro-neurovascular anatomical studies in laboratory mice, the most widely used animal models for *in vivo* studies in biomedical research, pharmaceuticals, gene therapy, and various other scientific fields. The mouse tail is the preferred site for intravenous administration, blood collection, intubation, and vascular anastomosis [5-8]. In previous studies, immunity-and-matrix regulatory cells (IMRCs) were injected via the tail vein to investigate the pathology of Alzheimer's disease (AD) [9], and tail vein injection has also been applied in research relating to chronic inflammatory diseases, including atherosclerosis [10]. In addition, the lateral tail vein is the most commonly used route for delivering agents or radiotracers in small-animal PET imaging [11]. Given its widespread use, a detailed understanding of mouse tail vascular anatomy is essential. In recent years, the advancement of supermicrosurgery has enabled precise microneurovascular anatomical studies in laboratory mice [12]. More recently, research using silicone perfusion has identified addition-

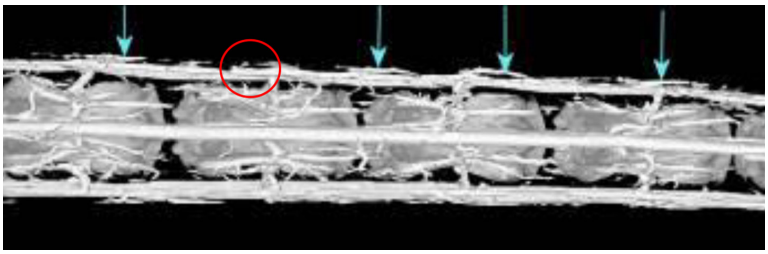

Figure 12. Micro-CT scanning of an artery in a mouse tail. The blue arrowhead indicates the caudal cutaneous artery.

Table 1. Abbreviation table

Abbreviation	Full Name
MCA	The middle caudal artery
LCA	Lateral caudal artery
DCA	The dorsal caudal artery
DCA5	Dorsal caudal artery-5
TCA	Transverse caudal artery
LLVTCA	Left lateral ventral transverse caudal artery
RLVTCA	Right lateral ventral transverse caudal artery
LLDTCA	Left lateral dorsal transverse caudal artery
RLDTCA	Right lateral dorsal transverse caudal artery
LLVTCA5	Left lateral ventral transverse caudal artery-5
DeCA	Deep caudal artery
LVDeCA	Left ventral deep caudal artery
RVDeCA	Right ventral deep caudal artery
LDDeCA	Left dorsal deep caudal artery
RDDeCA	Right dorsal deep caudal artery
VCCA	Ventral caudal cutaneous artery
LCCA	Left caudal cutaneous artery
RCCA	Right caudal cutaneous artery
DCCA	Dorsal caudal cutaneous artery

al tiny peripheral vessels. In the present study, we applied various technical methods and further demonstrated the intricate structure of the vasculature in the mouse tail, mapped the complete network of blood vessels, and proposed a comprehensive naming system for these vessels.

In this study, we identified, for the first time, the locations and course of the transverse caudal artery and vein, the deep caudal artery and vein, and the dorsal deep caudal vein valve. We also identified two blood circulation systems in the tail vessels of mice: the longitudinal blood circulation system, which encompasses the entire tail, and the local circulation system; crucially, we also identified structural connections between these two systems. The longitudinal circulatory system included three groups of blood vessels: the middle caudal vessels and the left and right caudal vessels, which provide the blood supply to the tail. The longitudinal circulatory system has specific characteristics: (1) superficial: all vessels run subcutaneously; (2) penetration: the middle caudal artery and vein represent continuations of the middle sacral artery and vein, reaching a few millimeters to the tip of the tail; (3) size: these three groups of arteries and veins represent the largest vessels in the tail; (4) two groups of transverse caudal arteries and veins branched out into the middle of each caudal vertebrae; and (5) unidirectional blood flow: all blood flow in the arteries originates at the root of the tail to the tip of the tail; the veins run in the opposite manner.

The local circulatory system takes the caudal vertebrae as a unit, and each caudal vertebrae is surrounded by a circulatory system. The arterial blood originates from the transverse caudal artery. The transverse caudal artery branches out into anterior and posterior branches, respectively. The corresponding vessels of the adjacent caudal vertebrae units connect to form the longitudinal deep caudal artery and dorsal caudal artery, with specific characteristics: (1) these blood vessels are smaller than those in the longitudinal circulatory system; (2) the caudal vertebrae is the unit for local circulation; (3) the dorsal deep caudal artery and vein represent longitudinal vessels, and the blood flow in the artery originates from the middle of the caudal vertebrae to the intervertebral space of the tail with the vein running in the opposite direction; (4) the transverse caudal artery originates from the middle caudal artery. The lateral caudal artery originates from the middle of each cau-

dal vertebrae, runs deep under the tail muscle, and produces longitudinal anterior and posterior branches of the dorsal deep caudal artery, respectively; these are anterior and posterior to the corresponding vessels of the adjacent caudal vertebrae units.

We were unable to demonstrate whether the direction of blood flow in the dorsal caudal artery and vein was different from that of the middle caudal artery and the lateral caudal artery and vein, as described in previous publications. Our present analysis demonstrated that the lateral caudal artery and vein are components of the local blood circulation.

There are two types of blood circulation in the mouse tail; collectively, these systems enable specific physiological functionality, predominantly compensatory and protective functions. When the longitudinal blood vessels are blocked, the local circulatory vessels compensate and dilate, thus forming a new vascular pathway. For example, when the lateral caudal vein is blocked on one side, then the dorsal transverse vein on the healthy side will dilate, thus connecting the distal transverse vein at the distal end of the blocked side. This allows blood to flow from the affected side into the healthy side and back to the heart. Due to the existence of local circulation, an obstruction in the local blood circulation in a single caudal vertebra will not influence the blood supply of other caudal vertebrae.

By investigating the tail vessels of mice, it will be possible to optimize tail-related experimental procedures according to specific characteristics of the tail vessels. For example, scientists often inject material intravenously directly into the tail. Inserting a needle between two caudal vertebrae can effectively avoid injury to the transverse caudal artery and vein. Another potential application is the specific selection of the site for end-to-end anastomosis in the middle caudal artery. For example, selecting the distal tail space of the 10th caudal vertebrae could avoid interference with the lateral sacral artery, collateral caudal artery and transverse caudal artery. Our data could help to select specific sites for blood collection from the lateral/middle caudal artery and vein; inserting a needle into the intervertebral space in the tail could help to avoid injury to the transverse caudal artery. Finally, when snipping tails for blood collection, enhanced anatomical knowledge would facilitate snipping of the tail containing blood vessels and not the vertebrae.

Conclusion

Our findings provide the first detailed characterization of the deep vascular system in the mouse tail and demonstrate that the dorsal caudal vessels play a crucial role in local blood circulation in the tail. Advances in anatomical research form the cornerstone of experimental animal studies, which, in turn, are fundamental to all aspects of *in vivo* research. To our knowledge, this is the first study to establish a systematic naming framework for transverse caudal vessels and deep caudal vessels, offering valuable insights for future investigations.

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Disclosure of conflict of interest

None.

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