The para-aortic ridge plays a key role in the formation of the renal, adrenal and gonadal vascular systems.

**Results**

Para-aortic ridge

**Materials and methods**

We adopted the micro-resin casting technique (Isogai & Horiguchi, 1996) to visualize the 3D arterio-venous vascular networks in the developing kidney. The perfusion of these vessels. A mixture of 30% (v/v) methyl, 17.5% (v/v) N, N-dimethylaniline (Nacalai Tesque) and 75% (v/v) peroxide was prepared for every 0.5- and 1-day interval during E14–E15 and E15–E20, respectively, as described above for the dye injection procedure. The umbilical vein was incised under a stereomicroscope to enable the removal of the blood and then with 2% glutaraldehyde to fix the specimen. The perfused specimens were stored temporarily in 30% (v/v) sodium cacodylate buffer (pH 7.4) and then rinsed with 0.1 M PBS for 2 h at 4°C, rinsed with 0.1 M PBS and post-fixed with 1% OsO4 in PBS for 2 h at 4°C. They were dehydrated with 50, 70, 80, 90 and 100% ethanol, and then frozen in 100% t-butanol. The specimens were coated with osmium (Filgen OPC 60A) before observation with an SEM (Hitachi S-4700) using an acceleration voltage of 15 kV.

Several non-injected embryos at each developmental stage were fixed prior to SEM observation. The embryos were rinsed with 0.1 M phosphate buffer, pre-fixed with 2.5% glutaraldehyde (v/v) and post-fixed with 1% OsO4 in PBS for 2 h at room temperature. The embryos were rinsed with PBS several times, incubated with secondary antibody (Alexa Fluor 594 goat anti-mouse IgG), which had been diluted (×200) with PBS-ST 0.1%, for 60 min at room temperature. The embryos were then rinsed with PBS and 0.1 M acetic acid in PBS and mounted on stubs. The secondary antibody conjugated to FITC or Texas Red was visualized with a fluorescence microscope (Olympus BX51, Tokyo, Japan).

**Figure 1**

A micrograph showing the arterial network in a dissected embryonic day 14.5 (E14.5) kidney with the striata arteriae (asterisks) and primary renal arteries (arrows). The renin-angiotensin system is induced in the primary renal arteries and star-shaped cells (white arrowheads) are stained positive for renin. White scale bar: 100 μm.

**Figure 2**

A micrograph showing the arterial network in a dissected embryonic day 14.5 (E14.5) kidney with the renin-angiotensin system (stars) and primary renal arteries (arrows). The renin-angiotensin system is induced in the primary renal arteries and star-shaped cells (white arrowheads) are stained positive for renin. White scale bar: 100 μm.

**Figure 3**

A micrograph showing the arterial network in a dissected embryonic day 14.5 (E14.5) kidney with the renin-angiotensin system (stars) and primary renal arteries (arrows). The renin-angiotensin system is induced in the primary renal arteries and star-shaped cells (white arrowheads) are stained positive for renin. White scale bar: 100 μm.

**Figure 4**

A micrograph showing the arterial network in a dissected embryonic day 14.5 (E14.5) kidney with the renin-angiotensin system (stars) and primary renal arteries (arrows). The renin-angiotensin system is induced in the primary renal arteries and star-shaped cells (white arrowheads) are stained positive for renin. White scale bar: 100 μm.

**Figure 5**

A micrograph showing the arterial network in a dissected embryonic day 14.5 (E14.5) kidney with the renin-angiotensin system (stars) and primary renal arteries (arrows). The renin-angiotensin system is induced in the primary renal arteries and star-shaped cells (white arrowheads) are stained positive for renin. White scale bar: 100 μm.

**Figure 6**

A micrograph showing the arterial network in a dissected embryonic day 14.5 (E14.5) kidney with the renin-angiotensin system (stars) and primary renal arteries (arrows). The renin-angiotensin system is induced in the primary renal arteries and star-shaped cells (white arrowheads) are stained positive for renin. White scale bar: 100 μm.

**Figure 7**

A micrograph showing the arterial network in a dissected embryonic day 14.5 (E14.5) kidney with the renin-angiotensin system (stars) and primary renal arteries (arrows). The renin-angiotensin system is induced in the primary renal arteries and star-shaped cells (white arrowheads) are stained positive for renin. White scale bar: 100 μm.

**Figure 8**

A micrograph showing the arterial network in a dissected embryonic day 14.5 (E14.5) kidney with the renin-angiotensin system (stars) and primary renal arteries (arrows). The renin-angiotensin system is induced in the primary renal arteries and star-shaped cells (white arrowheads) are stained positive for renin. White scale bar: 100 μm.

**Figure 9**

A micrograph showing the arterial network in a dissected embryonic day 14.5 (E14.5) kidney with the renin-angiotensin system (stars) and primary renal arteries (arrows). The renin-angiotensin system is induced in the primary renal arteries and star-shaped cells (white arrowheads) are stained positive for renin. White scale bar: 100 μm.

**Figure 10**

A micrograph showing the arterial network in a dissected embryonic day 14.5 (E14.5) kidney with the renin-angiotensin system (stars) and primary renal arteries (arrows). The renin-angiotensin system is induced in the primary renal arteries and star-shaped cells (white arrowheads) are stained positive for renin. White scale bar: 100 μm.