Myocardial interstitial Cajal-like cells (ICLC) in caveolin-1 KO mice

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Abstract

We compared, by transmission electron microscopy (TEM), the ultrastructure of interstitial Cajal-like cells (ICLC) in normal mammalian myocardium *versus* caveolin-1 null mice. TEM showed that myocardial ICLCs of caveolin-1-deficient mice retain their main ultrastructural characteristics, for example, location among cardiomyocytes, close vicinity to nerves and/or blood capillaries, specialized cell-to-cell junctions, presence of 2–3 typical processes, which are very long (several tens of micrometres), but are very thin (0.1–0.2 μm) and moniliform. However, the most striking modification of myocardial ICLC in caveolin-1 KO mice was *the absence of caveolae*. Beyond this main observation, three other findings could be reported: (1) the absence of caveolae in capillary endothelium, (2) persistence of (some) caveolae at the level of cardiomyocte sarcolemma or vascular smooth muscle cell sarcolemma and (3) (un)expected ultrastructural modifications such as increased thickness of capillary basement membrane and increased autophagy of several cardiomyocytes.

Keywords: interstitial Cajal-like cells (ICLC) • caveolae • caveolin-1 null mice • myocardium • autophagy • heart failure • blood capillary • basement membrane • endothelium

Caveolae are found in many cell types and are involved in a variety of cellular processes (reviewed in [1–10]). Caveolin-1 is the principal protein of caveolae, and therefore, caveolin-1 knockout mice were extensively used to identify the presumptive functions of caveolae in health and disease (reviewed in [11–13]). Moreover, cardiovascular dysfunctions were targeted in caveolin-1 null mice, and indeed, the ablation of caveolin-1 resulted in severe disorders, including an evident cardiomyopathy [14–24].

Interstitial Cajal-like cells (ICLC) were described in human and mammalian myocardium since 2005 [26–31] as a novel cell type, different from fibroblasts. ICLCs have specific ultrastructural characteristics and immunophenotypical features that enable their unequivocal identification ('platinum standard' [27–29]). To our knowledge, the ultrastructure of ICLC was not yet examined in caveolin-1 null mice.

Hearts from two Cav-$1^{+/+}$ (B6129PF2/J) and two Cav-$1^{-/-}$ (Cav-1 KO; Cav1 tm1Mls/J) 10-week-old mice purchased from Jackson Laboratories (Bar Harbor, ME, USA) were examined by transmission electron microscopy (TEM). The institutional ethical committee approved the study. Small fragments from atrial and ventricular myocardium were processed according to routine procedures, as previously described [27]. Ultrathin sections were examined using a Morgagni TEM (FEI Company, Eindhoven, Nederland), and images were recorded with a MegaView III CCD using iTEM-SIS software (Olympus, Soft Imaging System GmbH, Münster, Germany). To make ICLCs more evident, the TEM images (Figs. 1–3) have been digitally coloured using Adobe Photoshop (Adobe Systems Inc., San Jose, CA, USA). The colour codes are ICLCs-blue, endothelial cells – brown and nerve endings – light green.

Figure 1 shows the presence of typical caveolae along a long process of an ICLC in the myocardium of control mice. Note the moniliform aspect due to dilated portions, containing caveolae, endo(sarco)plasmic reticulum and/or mitochondria, the so-called 'Ca^{2+}-handling units' [5, 32–37].

Figures 2–5 show that ICLCs of caveolin-1 KO mice retain their ultrastructural organization, except the lack of caveolae. This could impair the Ca^{2+} signalling capability of ICLC, because, as it is mentioned above, caveolae are a key player in Ca^{2+} handling. We confirm here that endothelial cells of caveolin-1 KO mice lose their caveolae (Figs. 2, 3 and 5A). However, vascular smooth muscle cells and cardiomyocytes display (some) caveolae (Figs. 2, 3B and 4), because caveolin-1 is present in normal cardiomyocyte plasma membrane [38]. Noteworthy, we found capillaries with thickened laminated basement membranes (Fig. 5A). In addition, we observed numerous large autophagosomes in several cardiomyocytes (Fig. 5B and C) and a high number of macrophages in the interstitium. Autophagy may cause limited survival of cardiomyocytes [39], but the entire set of ultrastructural modifications found in the myocardium of caveolin-1 KO mice could explain the heart failure [21, 24, 40].

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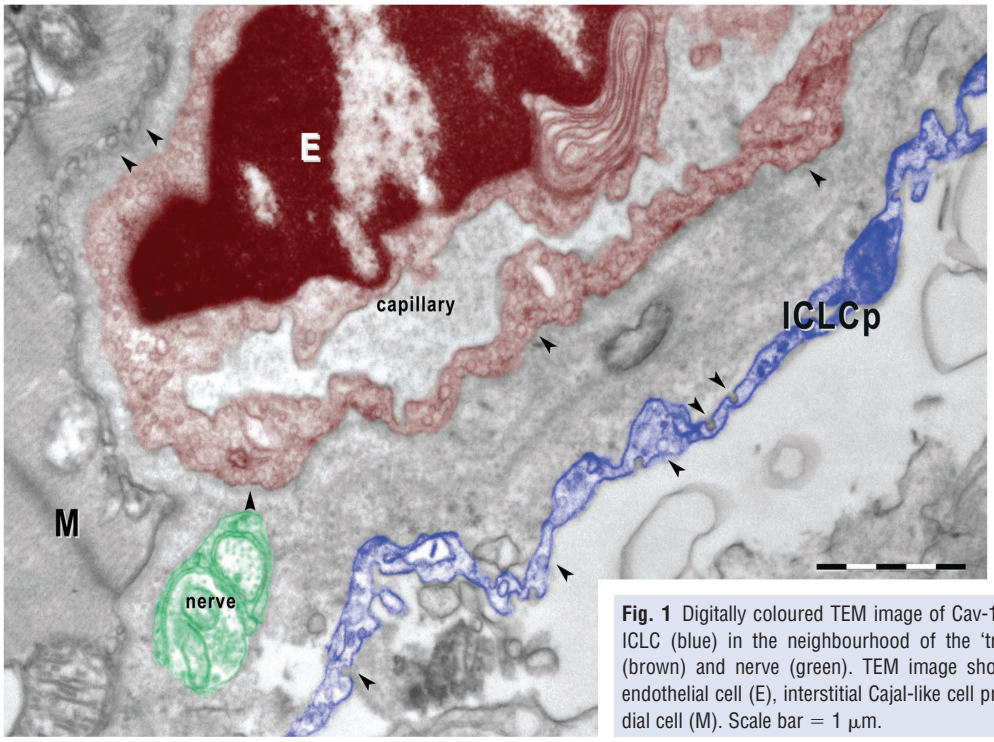


Fig. 1 Digitally coloured TEM image of Cav-$1^{+/+}$ mouse myocardium. The ICLC (blue) in the neighbourhood of the 'trio': myocardial cell, capillary (brown) and nerve (green). TEM image shows caveolae (arrowheads) in endothelial cell (E), interstitial Cajal-like cell processes (ICLCp) and myocardial cell (M). Scale bar = 1 μm.

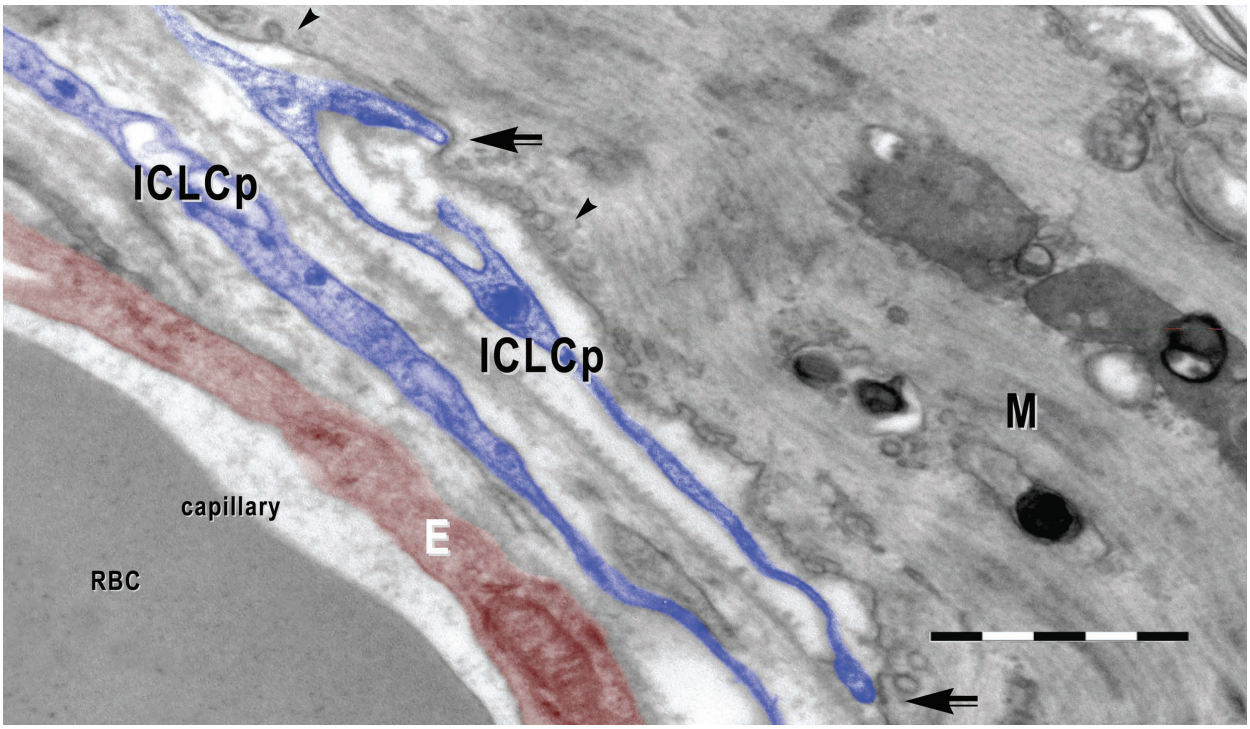


Fig. 2 Digitally coloured TEM image of the myocardium in Cav-$1^{-/-}$ (Cav-1 KO) mouse. In Cav-1 KO mice, no caveolae could be seen in the endothelium (E, brown) or in the ICLC processes (ICLCp, blue). The ICLCp are located in between the blood capillary and myocytes. However, several caveolae could be seen on the myocyte membrane (arrowheads). Note close contacts (arrows) between ICLCp and myocyte (M). Scale bar = 1 μm.

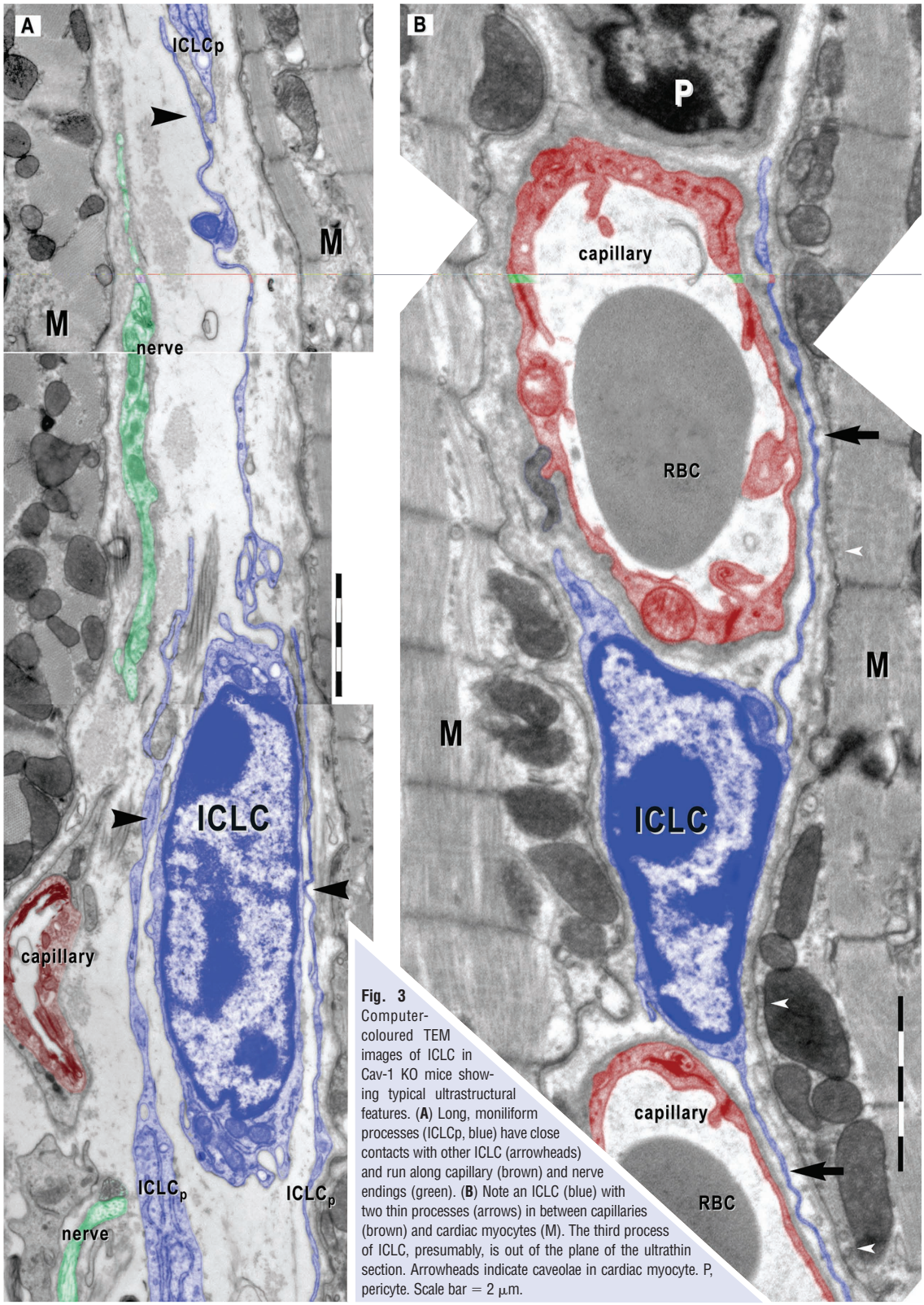


Fig. 3 Computer-coloured TEM images of ICLC in Cav-1 KO mice showing typical ultrastructural features. (**A**) Long, moniliform processes (ICLCp, blue) have close contacts with other ICLC (arrowheads) and run along capillary (brown) and nerve endings (green). (**B**) Note an ICLC (blue) with two thin processes (arrows) in between capillaries (brown) and cardiac myocytes (M). The third process of ICLC, presumably, is out of the plane of the ultrathin section. Arrowheads indicate caveolae in cardiac myocyte. P, pericyte. Scale bar = 2 μm.

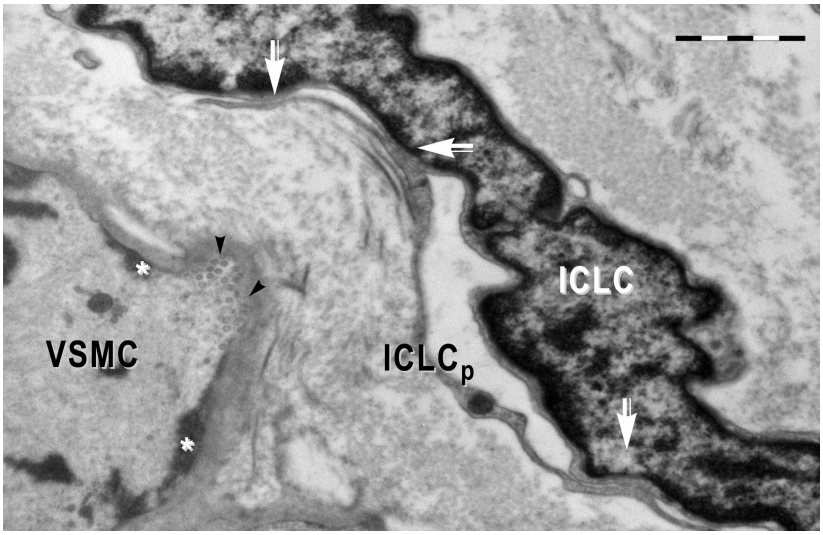
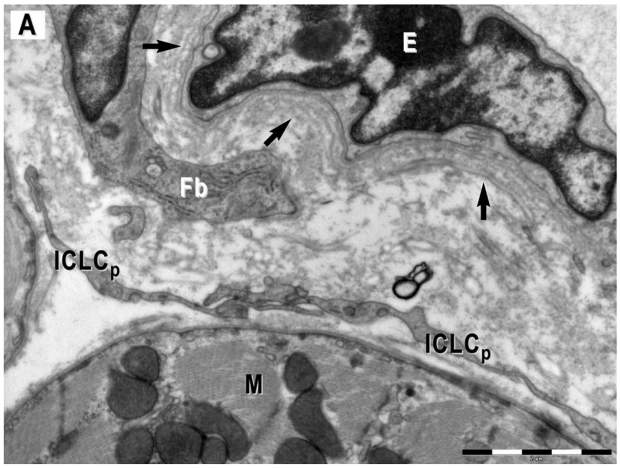
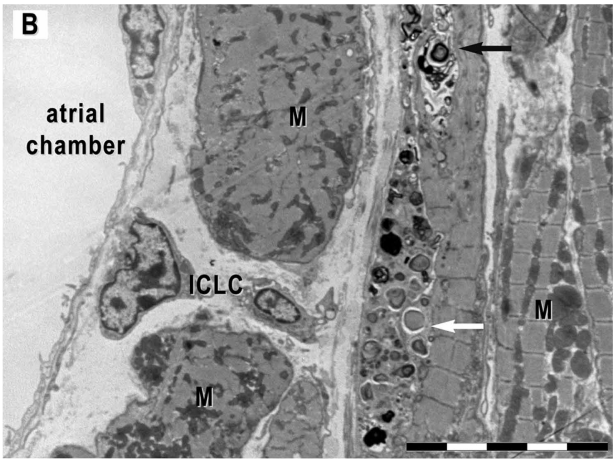


Fig. 4 TEM image of the Cav-1 KO mice heart shows that ICLCs form a network. Note close contacts between ICLC processes (ICLCp) and the cellular body of another ICLC (arrows). Caveolae (arrowheads) could be seen in vascular smooth muscle cells (VSMC), between the dense areas on the inner side of the sarcolemma (asterisk). Scale bar = 1 μm.





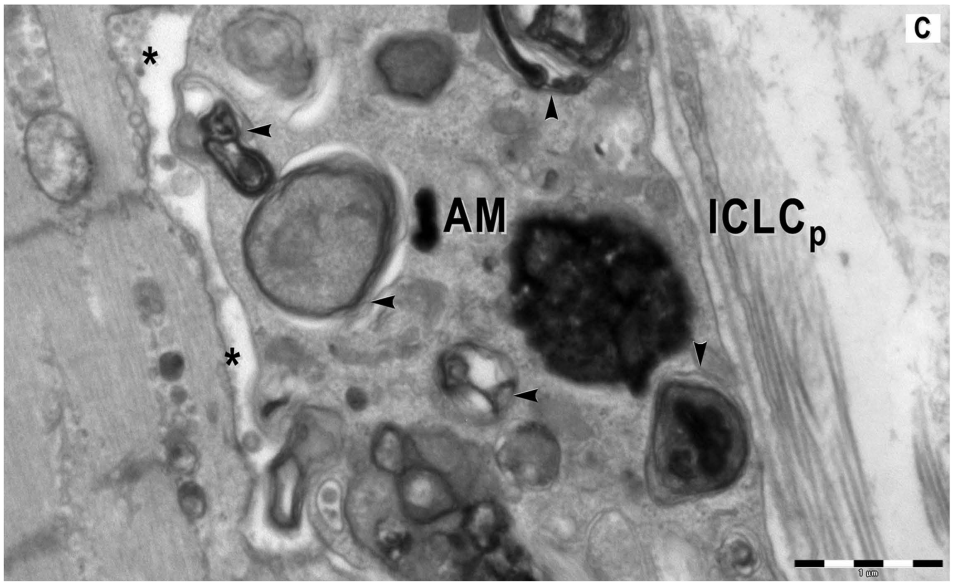


Fig. 5 TEM images of the Cav-1 KO mice heart. (**A**) Note the thickened and laminated basement membrane (arrows) of the blood capillary. M, myocyte; E, endothelial cell; Fb, fibroblast; ICLCp, ICLC process. Scale bar = 2 μm. (**B**) Extensive cytoplasmic assembly of autophagosomes in two cardiac myocytes (arrows). Scale bar = 10 μm. (**C**) High magnification of an autophagic myocyte (AM) exhibiting autophagosomes, loss of contractile elements and loose connection with adjacent myocyte (*). Arrowheads indicate the characteristic double membranes of the autophagosomes. Scale bar = 1 μm.

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