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Human epicardium: ultrastructural ancestry of mesothelium and mesenchymal cells

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Abstract

The human sub-epicardial area contains an unexplored cellular population under a layer of mesothelial cells. Transmission electron microscopy revealed the existence of interstitial Cajal-like cells (ICLCs), isolated smooth muscle cells (iSMC) and mesenchymal cells besides other well-known cells. The presence of iSMC in the sub-epicardial space is quite unique and could explain why epicardial-derived cells isolated from human epicardium generate smooth muscle cells *in culture*. Mesenchymal cells, guided by ICLCs, were found migrating from sub-epicardial area in the mesothelial layer. These findings suggest that epithelial-mesenchymal transition is not a common process involved in cardiac regeneration *in vivo*.

Keywords: (sub)epicardium • epithelial-mesenchymal transition (EMT) • isolated smooth muscle cells • interstitial Cajal-like cells (ICLCs) • mesothelial cells • mesenchymal cells • cardiac regeneration

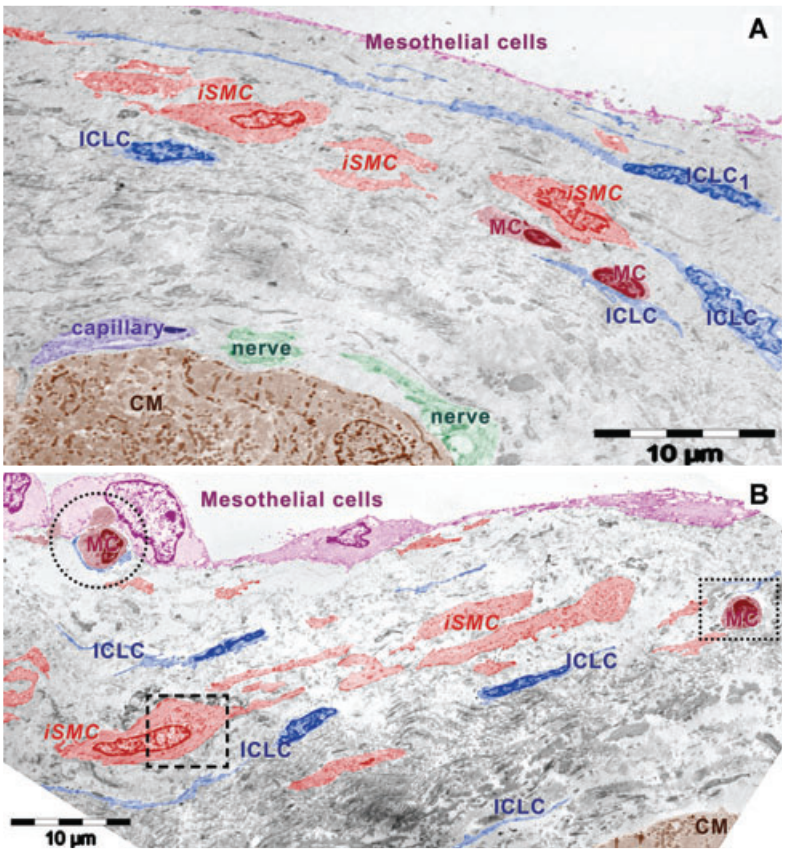

Fig. 1 (A), **(B)**. Digitally coloured electron microscope images of human epicardium emphasize the mesothelial cells (purple), ICLCs (blue), iSMC (orange), mononuclear mesenchymal cells (reddish), cardiac myocytes (brown) and nerves (green). The $ICLC_1$ in (**A**) has 40 μm length. (**B**) Details of marked cells in Fig. 2A (square dashed mark), Fig. 2B (square dot mark) and Fig. 3 (round mark).

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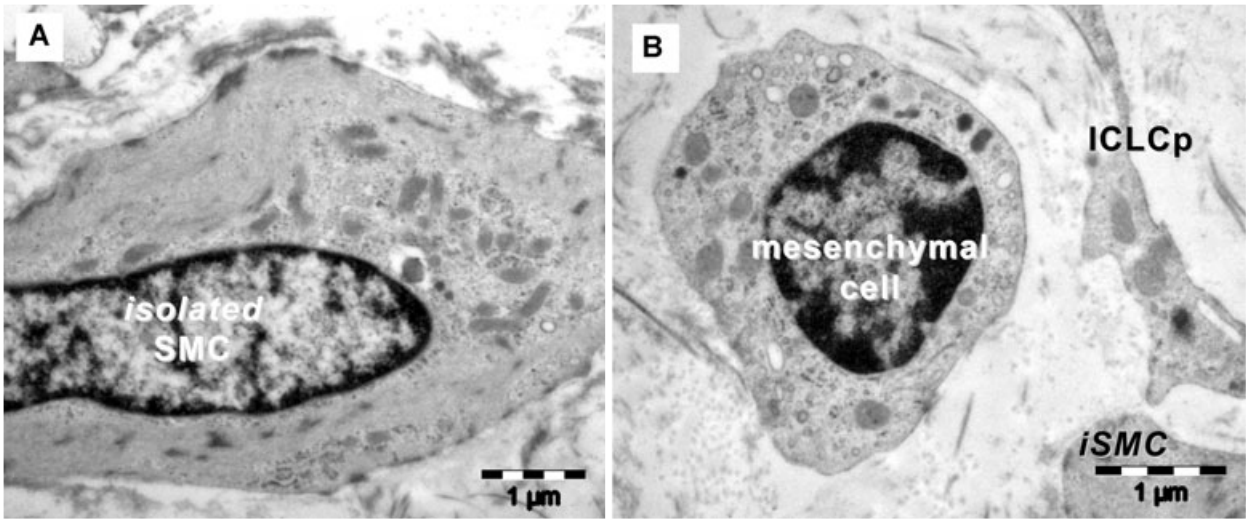

Fig. 2 (**A**) Isolated smooth muscle cell (iSMC) with organized cytoskeleton, dense bodies and centrally located mitochondria and sarcoplasmic reticulum. (**B**) Mesenchymal cell in the vicinity of an ICLC process and an iSMC.

Epithelial-mesenchymal transition (EMT) has been described primarily during embryonic development and it is essential in tissue remodelling and cell migration [1]. In the adult life EMT is associated with pathological conditions such as cancer progression, metastasis or fibrotic disorders [2, 3].

At the heart level, in the embryonic life, a sub-population of epicardial cells undergoes EMT and migrates into the myocardium with decisive role in the development of the coronary vascular system and myocardial differentiation [4, 5]. Recent papers suggest that EMT is also involved in cardiac regeneration and repair

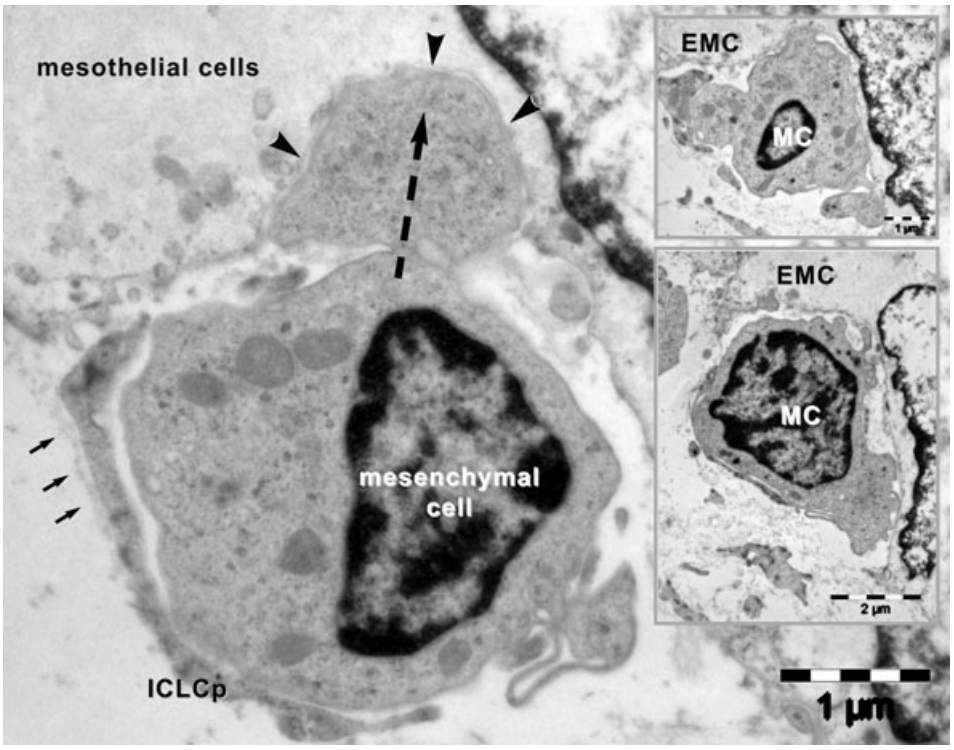

Fig. 3 Electron micrograph shows a mesenchymal cell migrating under the basal lamina (small arrows) of the epicardial mesothelial cell (EMC). Close plasma membranes contact (arrowheads) could be seen between the cellular process (dashed arrow) of the mesenchymal cell and mesothelial cell. An ICLC process (ICLCp) attends the mesenchymal cell. The insets show the same mesenchymal cell (MC) in serial sections.

[6–8]. Epicardial cells or epicardial-derived cells seem to preserve the ability to generate smooth muscle cells, endothelial cells or cardiomyocytes in response to specific growth factors [6–8].

Human right atrial appendage tissue was obtained from 10 patients undergoing cardiac surgery. Tissue samples were collected from patients who had given informed consent before surgery. Transmission electron microscopy was performed on small fragments of human myocardium with epicardium processed according to routine procedures, as we previously described [9–11].

We have found in the human sub-epicardial space interstitial Cajal-like cells (ICLCs), isolated smooth muscle cells (iSMC) and mesenchymal cells (Fig. 1) besides other well know cell (lymphocytes, mast cells, macrophages, fibroblasts, Schwann cells and nerves).

We have already described in human beings the ICLCs as new type of cells in atrium [9–11] and cardiac sleeves of the pulmonary veins [12] and in the mice myocardium [13, 14], epicardium [15] and cardiac stem cell niches [16]. The ICLCs seem to make a 3D network connecting heterogeneous cell types into the heart.

The presence of iSMC (Figs. 1 and 2A) in the sub-epicardial space is quite unique and it could explain why cells isolated from human epicardium generate smooth muscle cells *in culture*. Small mononuclear cells with mesenchymal features could be often observed in the sub-epicardium (Figs 1 and 2B) or in the myocardial interstitium. Mesenchymal cells contained a slightly indented, heterochromatic nucleus surrounded by a moderate amount of cytoplasm that contained mitochondria, few endoplasmic reticulum cisternae, intermediate filaments and small dense granules (Fig. 2B).

Moreover, some images suggest that even mesothelial cells (epicardium) could be regenerated from these mesenchymal cells (Figs 1B and 3). Transmission electron microscopy showed mesenchymal cells extending small processes under basement membrane of the mesothelial cells and establishing close contacts with them (Fig. 3). These findings suggest that EMT is not a common choice for cardiac regeneration *in vivo*.

In a heart full of stem cells [17] these small round mesenchymal cells, guided by ICLCs, could be the resident or exogenous stem cells used for cardiac regeneration.

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