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TELOCYTE IDENTITY – A DISTINCT CELL OR A DIFFERENT PHENOTYPE [TIDY]

Brief scientific report – 2021

PHASE 1. *In silico* analysis to identify potential specific molecular markers for telocytes in tissue

Act. 1.1 Identification of specific databases – analysis of molecules with potential to act as markers for telocytes
Act. 1.2 Testing of potential telocyte-specific markers

Data included in the following open-access databases were analyzed to assess the potential of several proteins to act as markers for telocytes (TCs): The Human Protein Atlas (https://www.proteinatlas.org/); UniProt (https://www.uniprot.org/); STRING (https://string-db.org/). Based on this analysis and on the published literature, PDGFRα, Foxl1, Tcf21 and Nkx2-5 were selected for experimental testing.

Initially, immunofluorescence experiments for TC phenotyping were performed on heart harvested from two C56Bl/6J mice, aged 6 months. Mice were anesthetized before sacrifice according to standard protocols. The heart was removed and after a preliminary wash with saline solution it was mounted on a cryotome stand in cryoprotection gel. Sections with a thickness of 5 µm were obtained parallel to the long axis of the heart to contain both the atrium and ventricle and then fixed for 5 min in cold acetone. Blocking of nonspecific sites was performed by incubating the sections with 1% bovine serum albumin (BSA) for 15 min. Immunolabeling was performed by the indirect method, with a set of primary antibodies against the marker proteins of interest and then with corresponding secondary antibodies, conjugated with AlexaFluor 568 (red) or AlexaFluor 488 (green). Specifically, the primary antibodies tested as TC markers were anti-CD34 and anti-PDGFRα, whereas anti-procollagen I antibodies were used for fibroblast-specific labeling. Additionally, anti-CD45 and anti-nestin antibodies were used to label cells of the hematopoietic lineage or nerves. Nuclei were stained with 1.0 µg/mL 4',6-diamidino-2-phenylindole (DAPI, blue emission). Negative controls were performed following the same protocol but excluding the primary antibody and incubating with non-immune sera.

Immunofluorescence examination of samples and image acquisition was performed on a Leica epifluorescence (wide-field fluorescence) microscope.

These immunofluorescence experiments revealed non-specific binding for CD34. The antibody used (sc-7045, C-18) bound to muscle cells in the vascular wall and cardiomyocytes. CD45 was present in restricted cell groups located pericardially at the atrial level, however the presence of stem cells in this region of the heart may explain this result. In these experiments, nestin was not detected in the heart specimens.

PDGFRα was detectable on small cells, but also on a population of cells with very obvious, lengthy extensions, likely TCs. PDGFRα did not colocalize with procollagen, which suggests either that PDGFRα-positive cells do not synthesize collagen or that they may synthesize collagen in pathological conditions or as part of the aging process. The 6-month-old mouse heart does not show abundant interstitial collagen.