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

Brief scientific report – 2022

PHASE 2. Identification and validation of specific markers for telocytes

Act. 2.1 Identification of a specific marker for telocytes
Act. 2.2 Analysis of distribution of telocytes in the adult mouse heart

In this phase, immunofluorescence and immunoelectron microscopy assays for telocyte (TC) phenotyping were performed on hearts harvested from 6-month-old C56Bl/6J (normal control) and PDGFRα-EGFP mice. Mice were anesthetized before sacrifice according to the standard protocol of the Victor Babes Institute animal facility. The cardiac tissue was specifically processed for either optical microscopy, immunofluorescence, immuno-electron microscopy or electron microscopy.

Primary antibodies were tested against molecules selected to have the highest potential of being TC-specific, in accordance to the analysis in Phase 1 of this project.

The following antibodies were tested by immunofluorescence:

primary antibody	#	species	dilution	secondary antibody
PDGFRα	sc-338 (santa cruz)	rabbit (R)	1/20	GAR 488
PDGFRα	ab203491 (abcam)	rabbit (R)	1/500	GAR 488
Foxl1	ab190226 (abcam)	rabbit (R)	1/100	GAR 488
Nkx2.5	ab97355 (abcam)	rabbit (R)	1/100	GAR 488
calreticulin	ab195511 (abcam)	rabbit (R)	1/800	GAR 488
Nestin	ab6142 (abcam)	mouse (M)	1/200	GAM 488
CD34	sc-7045 (santa cruz)	goat (G)	1/50	DAG 546
Procollagen 1	sc-25973 (santa cruz)	goat (G)	1/50	DAG 546
CD45	ab25386 (abcam)	rat (R)	1/150	DAR 546

Immunolabeling demonstrated that CD45 had focal distribution, in small cell clusters, possibly immune cells, without co-localization of PDGFRα. The anti-PDGFRα antibody (sc-338) labeled small cell groups. Among the PDGFRα-positive cells only a small fraction also showed positivity for CD34, however these were round and lacked the long extensions characteristic for TCs. No cells positive for Foxl1 and Nkx2-5 were identified, suggesting these proteins are not useful markers for cardiac interstitial cells, at least not in the murine heart.

To confirm the presence and distribution of PDGFRα positive cells we also used a recombinant rabbit monoclonal anti PDGFRα [EPR22059-270] antibody (ab203491). This antibody showed higher specificity, less background and better signal, which allowed the analysis of labeled sections at higher resolution, using confocal microscopy.

This antibody highlighted the presence of interstitial cells with long network-forming extensions, suggestive of TCs. These elongated, PDGFRα-positive cells are located in perivascular regions, around the coronary and medium-caliber arteries, which matches the known distribution of TCs. Importantly, this antibody did not visibly label cells deep in the ventricular or atrial myocardium, at distance from the great vessels.

Immuno-electron microscopy tests performed by the post-embedding labeling technique did not yield the expected results when attempting PDGFRα labeling. This was either due to hindered antibody activity on specimens embedded in the hydrophilic resin LR White, or to denaturation of PDGFRα by thermal and/or chemical processing of these samples. In the next phase, we will use pre-embedding or Tokuyasu techniques for immunolabeling for electron microscopy to circumvent these limitations.

The study demonstrates that PDGFRα is a strong cadidate for specific labeling of TCs. This conclusion is supported by publicly available transcriptomic data of the mouse heart (Tabula muris). This current phase of the study therefore confirms the presence and distribution of TCs, as PDGFRα-positive cells, preferentially positioned around arteries, extending into the septa between cardiomyocyte bundles.

Dissemination:

Dermal Telocytes: A Different Viewpoint of Skin Repairing and Regeneration. Cells 2022; 11(23), 3906; https://doi.org/10.3390/cells11233906. FI - 7,666.

Telocytes in cardiovascular system. Mihaela Gherghiceanu. Presentation at the „International Pathology Conference of The Victor Babeș Institute Bucharest, 2022", 17-19 November 2022, online.