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Involvement of Urokinase-Type Plasminogen Activator Receptor in the Formation of a Profibrotic Microenvironment in the Epicardial Region

Konstantin V. Dergilev[1]*, Zoya I. Tsokolayeva[1,2], Irina B. Beloglazova[1], Yuliya D. Vasilets[1], Dmitry O. Traktuyev[3], Boris N. Kulbitsky[4,5], Elena V. Parfenova[1,6]

[1] Angiogenesis Laboratory, Experimental Cardiology Institute,
National Medical Research Center for Cardiology, Ministry of Health of Russia,
15a Cherepkovskaya 3rd Str., 121552 Moscow, Russia
[2] V. A. Negovsky Research Institute of General Reanimatology,
Federal Research and Clinical Center of Intensive Care Medicine and Rehabilitology,
25 Petrovka Str., Bldg. 2, 107031 Moscow, Russia
[3] Center for Regenerative Medicine, Department of Medicine, College of Medicine, University of Florida,
1600 SW Archer Rd, M421 Gainesville, FL 32610 USA
[4] Pathology of Terminal States Section, Forensic Medicine Department, Research Institute of Human Morphology,
3 Tsyurupy Str., 117418 Moscow, Russia
[5] Forensic Medicine Department, Peoples' Friendship University of Russia,
6 Miklukho-Maclaya Str., 117198 Moscow, Russia
[6] Laboratory of Postgenomic Technologies in Medicine, Fundamental Medicine Faculty,
Lomonosov Moscow State University,
27 Lomonosovsky Avenue, Bldg.1, 119192 Moscow, Russia

Участие рецептора активатора плазминогена урокиназного типа в формировании профиброзного микроокружения в эпикардиальной области

К. В. Дергилев[1]*, З. И. Цоколаева[1,2], И. Б. Белоглазова[1], Ю. Д. Василец[1], Д. О. Трактуев[3], Б. Н. Кульбицкий[4,5], Е. В. Парфенова[1,6]

[1] Лаборатория ангиогенеза, Институт экспериментальной кардиологии,
Национальный медицинский исследовательский центр кардиологии Минздрава России,
Россия, 121552, г. Москва, ул. 3-я Черепковская, д. 15а
[2] НИИ общей реаниматологии им. В. А. Неговского ФНКЦ РР
Россия, 107031, г. Москва, ул. Петровка, д. 25, стр. 2,
[3] Центр регенеративной медицины, Медицинское отделение, Медицинский колледж, Университет Флориды,
США, Флорида 32610, Гейнсвилл, М421, 1600 SW Арчер рд
[4] Отдел патологии терминальных состояний кафедры судебной медицины, НИИ Морфологии человека,
Россия, 117418, г. Москва, ул. Цюрупы, д. 3
[5] Кафедра судебной медицины Российского университета дружбы народов,
Россия, 117198, г. Москва, ул. Миклухо-Маклая, д. 6
[6] Лаборатория постгеномных технологий в медицине, факультет фундаментальной медицины,
Московский Государственный университет им. М.В. Ломоносова,
Россия, 119192, г. Москва, Ломоносовский пр-т, д. 27, стр. 1

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Summary

The study of the mechanisms of development and progression of fibrosis is one of the key directions of modern cardiology. Our work suggests that the urokinase-type plasminogen activator receptor (uPAR) is involved in the regulation of mesothelial cell activity and epicardial fibrosis development, which, when interacting with specific ligands and intermediate proteins, can activate intracellular signaling, trigger the cascade of proteolytic reactions, including local plasmin formation and activation of matrix metalloproteinases, providing matrix remodeling.

Objective: to perform a comparative study of fibrogenic activity of the epicardium in the hearts of uPAR-/- and wild-type animals and evaluate the effect of cardiac microenvironment factors on the migration activity of epicardial mesothelial cells.

Material and methods. We used histological and immunofluorescent staining, microarray analysis of proinflammatory cytokine levels, and a method for assessing the migratory properties of epicardial cells.

Results. Results. We found that compared to wild-type animals, uPAR-/- animals show significant thickening of the epicardial area (2.46+0.77 (uPAR-/- mice) and 1.02+0.17 (Wt mice) relative units, *P*=0.033) accom-

Correspondence to:

*Konstantin V. Dergilev
E-mail: doctorkote@gmail.com

Адрес для корреспонденции:

*Константин Владимирович Дергилев
E-mail: doctorkote@gmail.com

panied by accumulation of extracellular matrix proteins. Deficiency of uPAR gene leads to formation of proinflammatory microenvironment in the heart (increased levels of proinflammatory factors such as IL-1, IL-13, IL-17, RANTES and MIP1), increased migratory activity of epicardial mesothelial cells, accumulation of TCF21+ fibroblast/myofibroblast precursors (29.8+13.7 (uPAR-/- mouse) and 3.03+0.8 (Wt mouse) cells per visual field, *P*=0.02), as well as development of subepicardial fibrosis.

Conclusion. These findings suggest that uPAR is a promising candidate for the developing targeted agents to prevent the development and progression of cardiac fibrosis.

Highlight

Deficiency of urokinase-type plasminogen activator receptor contributes to the formation of proinflammatory microenvironment and fibrogenic remodeling of epicardial area.

Keywords: fibrosis; epicardial mesothelium; urokinase receptor

Conflict of interest. The authors declare no conflict of interest.

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Introduction

Extracellular matrix (ECM) proteins are an important regulators of the structural organization of human heart, coordinating the efficient electromechanical coupling of myocardial cells, as well as forming a unique microenvironment to maintain the fundamental characteristics of cells and perform their reparative functions [1]. In a healthy heart, the balance of ECM components is maintained through their enzymatic degradation and de novo synthesis, which ensures the normal microenvironment homeostasis. However, when pathological conditions develop, this balance is disturbed, leading to excessive matrix deposition, known as cardiac fibrosis, which has a significant impact on cardiac function by increasing myocardial stiffness and impairing electrical conduction. Fibrosis of various organs is estimated to be directly or indirectly responsible for almost 45% of deaths in developed countries, which is highly important from a social point of view and carries an enormous economic burden on society [2, 3]. To date, there are no effective ways to reverse the pathological reorganization of the cellular microenvironment and influence the activity of fibroplastic processes in the heart, which inevitably leads to the development of severe heart failure and death. Therefore, the search for new biological targets and the study of the mechanisms of cardiac fibrosis development remains relevant. In this respect, attention has turned to the epicardium, the outer membrane-like layer of the heart, formed by a heterogeneous population of epicardial mesothelial cells and extracellular matrix proteins. Studies of transgenic mouse lines using Cre-lox homologous recombination targeting Wilms tumor genes 1 (Wt1) and Tcf21 revealed a population of progenitor cells in the epicardium that undergo epithelial-mesenchymal transition (EMT) during embryonic development and differentiate into a resident fibroblast line [4–8]. In the adult heart, when ischemia or pressure overload develop, the epicardial microenvironment remodeling occurs, leading to re-expression of fetal epicardial genes and fibroblast-like cell transformation [9–11].

This study suggested that urokinase-type plasminogen activator receptor (uPAR) may act as a regulator of epicardial microenvironment remodeling [12, 13]. It is an integral part of the urokinase system, which also includes urokinase (uPA) and two inhibitors (PAI-1 and PAI-2). uPAR is anchored in the cell membrane via the GPI anchor, which ensures its mobility in the membrane bilayer and allows it to focus the proteolytic activity of urokinase locally in the direction of cell movement. The cascade of proteolytic reactions triggered by urokinase, including local formation of plasmin and activation of matrix metalloproteinases, provides matrix remodeling. However, in addition to the activation of extracellular proteolysis, most cellular responses modulated by the urokinase system are enabled by transmembrane signaling, which is mediated by the interaction of components of this system with intermediary proteins, such as integrins.

Aim of the study: a comparative study of epicardial fibrogenic activity in the heart of uPAR-/- and wild-type animals and investigation of the influence of cardiac microenvironmental factors on the migratory activity of epicardial mesothelial cells.

Material and Methods

Animals. Male C57BL/129 mice (wild-type; n=20) and C57BL/129 uPAR gene knockout mice (uPAR-/- mice; *n*=20) donated by the Faculty of Fundamental Medicine, Lomonosov Moscow State University, were used in the study. The experiments were approved by the ethical committee of National Medical Research Center for Cardiology.

Detection of collagen fibers in the epicardial area. Visualization of collagen in the epicardial zone was done by staining the cryosections with pi-

crosirius red, according to the technique described in the literature [14].

Detection of TCF21+ fibroblast progenitor cells in the epicardial area. TCF21 cells were analyzed by immunohistochemical staining using a commercial ABC Elite Kit (Vector Lab, USA). Cryosections were thawed at room temperature (30 min), washed in phosphate-salt buffer (5 min), and fixed in 3.7% parapharmaldehyde solution (10 min). After fixation, the sections were washed with phosphate-buffered saline (PBS) (3 times for 5 minutes), permeabilized with 0.1% Triton X100 solution (5 minutes), endogenous peroxidase was blocked using the 3% H_2O_2 solution followed by washing with PBS. Next, the sections were blocked with a solution containing 1% bovine serum albumin (BSA) and 10% of the second antibody donor serum in PBS (30 min). After that, cryosections were stained with antibodies to TCF 21 marker (Biolegend, USA) for 1 hour. Afterwards, the slides were washed with PBS (3 times for 5 min each) and secondary biotinylated antibodies were applied to the sections for 30 min. Next, the slides were washed with PBS and treated with ABC kit for 30 minutes. The slides were then washed with PBS and stained with the substrate included in the DAB substrate kit. After staining, the slides were washed with distilled water, dehydrated, and mounted using xylene-based medium.

Obtaining epicardial mesothelial cell culture. The cells were isolated according to the protocol described earlier [15].

Assembly of spheroids based on epicardial mesothelial cells. To assemble epicardial spheroids, V-shaped cups with low-adhesion GravityTRAPTM ULA Plate were used. To obtain spheroids, a cell suspension (5000 cells in 70 µl of culture medium) was plated into wells, precipitated by centrifugation (200g, 2 min), and cultured for 72 hours (in IMDM medium supplemented with 1% fetal calf serum) under standard incubator conditions (37°C, 5% CO_2).

Evaluation of migratory properties of epicardial spheroid cells exposed to conditioned medium Wt uPAR-/- cardiac explants. Formed spheroids were placed in 48-well culture dishes with conditioned medium from Wt and uPAR-\- cardiac explants (½ of conditioned medium and ½ of IMDM medium without serum or other additives). Spheroids were cultured for 3 days with image recording every 24 hours. The migration area and migration pathway length were estimated using Image J software (NIH, USA)

Microarray analysis of proinflammatory factors secretion by Wt and uPAR-\- cardiac explants cells. Hearts were extracted from the thoracic cavity, large vessels were dissected out, and thoroughly washed in PBS. Next, the hearts were placed in sterile Petri dishes and crushed with scissors to obtain 1–2 mm slices. The obtained crushed heart samples were weighed and equalized by weight. Next, the crushed samples (explants) were planted in culture cups (uncoated) in IMDM medium (Gibco, USA) without additives and incubated at 37°C in a 5% CO_2 atmosphere. After 48 h, the explants were removed and the conditioned medium was centrifuged in 2 steps (1000g, 20 min). The resulting supernatant was aliquoted and stored at –70°C until proinflammatory cytokine studies and in vitro experiments were performed. The levels of inflammatory cytokines in conditioned media of cardiac explants of uPAR-/- mice and wild-type animals were studied using Mouse Inflammation Antibody Array (Abcam, USA) strictly according to the kit manufacturer's recommendations.

Statistical analysis. The normality of distribution of variables was assessed using the Kolmogorov–Smirnov test. Differences between the groups were assessed using Mann–Whitney *U*-test considering significance at $P<0.05$. Statistical analysis of the data was performed using Statistica 8.0 software (StatSoft, Inc.). The data were presented as mean±standard deviation (*M*±*SD*).

Results

In the hearts of 1-year-old animals knockout for the uPAR gene (uPAR-/- mice), collagen fiber accumulation was observed, which was combined with 2.4x thickening of the epicardial region (2.46±0.77 (uPAR-/- mice) and 1.02±0.17 (Wt mice) relative units, $P=0.033$), which was not found in wild-type animals (Fig. 1, *a, b, c*). Considering the identified changes, we assessed the number of fibroblast precursor cells in this area of the cardiac wall. The transcription factor Tcf21 was used to identify fibroblast precursors. This marker occurs in epicardial mesothelial and proepicardial cell populations and is involved in the regulation of differentiation toward fibroblast-like derivatives [7, 8]. We found that the number of TCF21+ cells was 9-fold higher (Fig. 1, *c, d, e*) in the epicardium/subepicardium of uPAR-/- mice compared with wild-type mice (29.8±13.7 and 3.03±0.8 cells per visual field, respectively; $P=0.02$).

To identify the factors that can initiate epicardial remodeling, we studied the levels of proinflammatory cytokines in conditioned cardiac explant media samples (Fig. 2, *a*), whose elevated levels are associated with the development of fibrosis. Increased levels of proinflammatory factors (IL-1, IL-13, IL-17, RANTES, and MIP1) were observed in uPAR-/- mice compared with control explant media (from wild-type mouse hearts).

Since thickening of the epicardial layer of the heart is associated with the loss of intercellular contacts and redistribution of mesothelial cells, we analyzed the influence of proinflammatory microenvironment factors on cell migration prop-

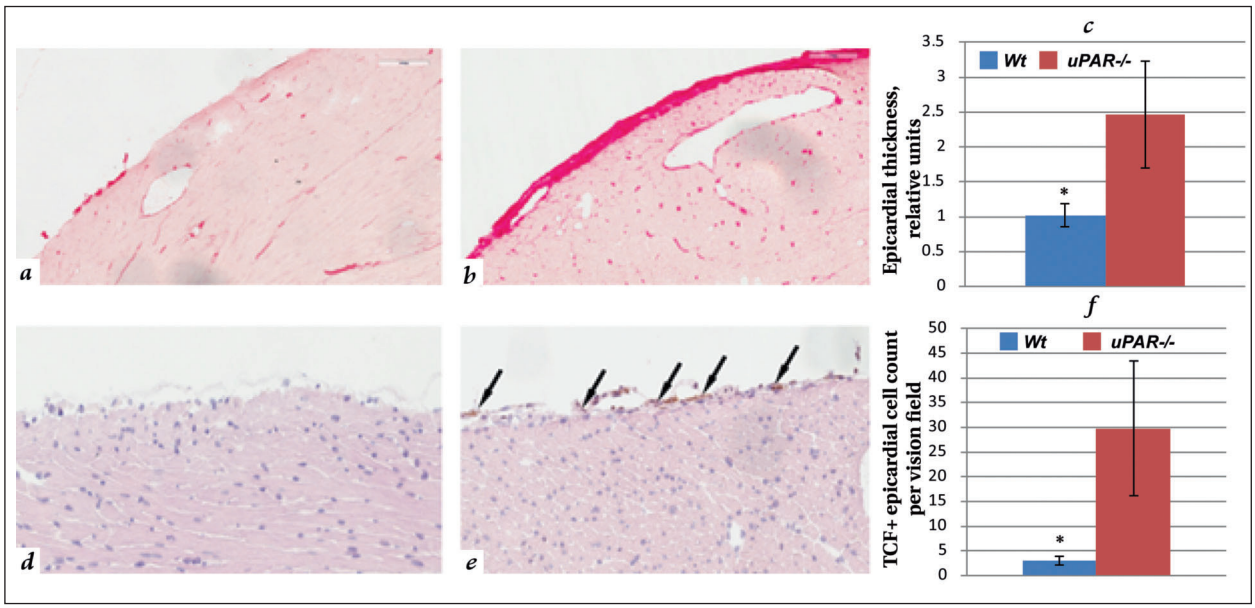

Fig. 1. Urokinase receptor deficiency is associated with epicardial thickening and increased number of TCF+ fibroblast progenitor cells.
Note. Representative staining of heart sections of wild-type (*a*) and uPAR-/- (*b*) mice with picrosirius red. *c* — quantification of epicardial zone thickness in wild-type and uPAR-/- mice. Representative staining of heart sections from wild-type (*d*) and uPAR-/- (*e*) mice with antibodies to the fibroblast progenitor cell marker, TCF21. f — quantification of TCF21+ fibroblast progenitor cells in the heart of wild-type and uPAR-/- mice. Data are presented as mean±standard deviation (*M*±*SD*). * — *P*<0.05.

erties. To study cell motility we used a 3D model of the epicardial microenvironment built according to the spheroid type that provided spatial interaction of cells and formation of cadherin intercellular contacts. Cell distribution area (day 3: 1069900±226137 (Wt explant medium) and 3329643±312000 (uPAR-/- explant medium; *P*=0.04) relative units) and maximum migration path length (day 3: 526±86 (Wt explants medium) and 987±57 (uPAR-/- explants medium) relative units; *P*=0.01) of the epicardial cells were significantly higher with conditioned media from uPAR-/- cardiac explants, compared with control media (Fig. 2, *b–d*).

Discussion

The studies showed that the absence of uPAR gene is associated with the formation of proinflammatory microenvironment in the heart, the accumulation of TCF21+ myofibroblast precursors, and the development of subepicardial fibrosis. Therefore, we hypothesize that uPAR is required to maintain the integrity of the cardiac epicardial layer and regulate the profibrogenic activity of mesothelial cells. uPAR is widely present in epithelium-like cells of different types; it is involved in tissue remodeling processes and participates in the regulation of the most important biological processes, including epithelial-mesenchymal transition, angiogenesis, fibrinolysis, inflammation, tumor invasion and metastasis [12, 13, 16]. In the absence of uPAR, urokinase system function is impaired, which is probably one of the reasons for the rearrangement of the epicardial/subepicardial microenvironment. Indeed, increased levels of proinflammatory factors were observed in the hearts of uPAR-/- mice, which may act as independent regulators of cellular function and underlie the development of fibrosis. A study by Genua [17] showed that uPAR deficiency causes polarization of macrophages in the M1 direction and promotes increased secretion of proinflammatory cytokines, which may act as the basis for the formation of a proinflammatory microenvironment. In the intact heart, the mesothelium has a polygonal epithelial-like morphology, but under the influence of inflammatory factors it may undergo transdifferentiation in the mesenchymal direction and acquires promigratory, proinvasive, and fibroblast-like characteristics. The transition from mesothelial to mesenchymal (fibroblast-like) phenotype in uPAR-/- animals may be based on the complex effect of inflammatory factors and altered activity of Ras-ERK1,2 MAPK, Rac1 and PI3K-AKT intracellular signaling pathways (due to impaired mutual influence of uPAR integrins or other intermediaries) [18, 19], which lead to disruption of intercellular contacts, cell polarity loss and cytoskeleton reorganization. Probably, the triggering of this irreversible reaction underlies the formation of fibroblasts/myofibroflasts hyperproducing extracellular matrix proteins, which are formed under the control of uPAR. The absence of the receptor leads to the inhibition of uPAR-dependent regulation of integrin functions and increased cell adhesion stimulating the transition of fibroblasts to myofibroblasts due to the formation of adhesion contacts and enhanced assembly/stabilization of smooth muscle alpha-actin fibers [20–22]. An additional unfavorable factor inducing the epicardial

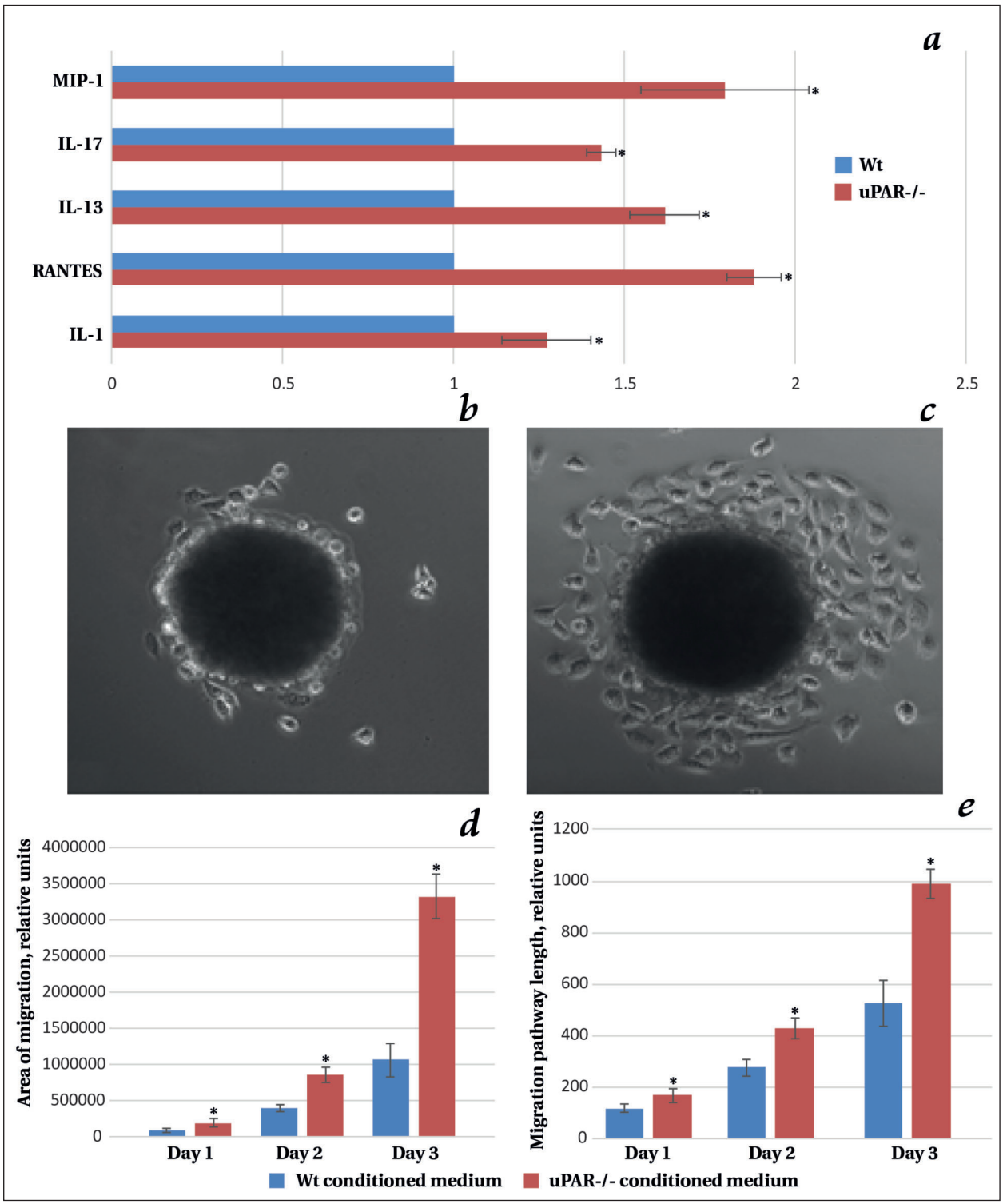

Fig. 2. Conditioned medium of uPAR-/- of cardiac explants is characterized by a high level of proinflammatory factors and has a stimulating effect on the migration properties of epicardial cells.
Note. *a* — quantification of proinflammatory factors in the medium of cardiac explants of wild-type and uPAR-/- mice; *b, c* — representative images of epicardial cell migration from spheroids under the influence of conditioned medium of cardiac explants of wild-type (*b*) and uPAR-/- mice (*c*); *d, e* — morphometric evaluation of cell distribution area and maximum migration pathway length when epicardial spheroids were cultured in conditioned medium of cardiac explants of wild-type and uPAR-/- mice. The data are presented as mean±standard deviation (*M*±*SD*). * — *P*<0.05.

microenvironment rearrangement can be accumulation of free (not related to uPAR) urokinase in the heart, which, through interaction with nucleolin, can be transported to nucleus to activate expression of EMT-associated and profibrotic genes [23, 24]. Another mechanism is possible that relates to the interaction of urokinase with alternative receptors, such as N-cholinoreceptors regulating fibrob-

last function [25] and fibrosis development/progression. The results obtained when studying uPAR-/- animals with signs of inflammatory microenvironment formation combined with cardiac fibrosis have common features with the clinical manifestations observed in patients with systemic scleroderma, a condition characterized by a loss of uPAR function due to its proteolytic cleavage by MMP12 [26]. Such patients have elevated levels of IL-1, IL-17, MCP-1/CCL2, MIP-1α/CCL3, MIP-1β/CCL4 and IL-8/CXCL8, accompanied by increased EMT activity, excessive fibroblast formation and fibrotic transformation of various tissues, including the heart [27, 28].

Conclusion

Thus, uPAR can be considered as a multilevel regulator of epicardial microenvironment. Deficiency of this gene leads to the formation of proinflammatory microenvironment in the heart, increased migratory activity of epicardial mesothelial cells, accumulation of TCF21+ fibroblast/myofibroblast precursors and development of subepicardial fibrosis. These data allow us to consider uPAR a promising candidate for the developing targeted agents to prevent the emergence and progression of cardiac fibrosis.

References

1. *Derrick C.J., Noël E.S.* The ECM as a driver of heart development and repair. *Development*. 2021; 148 (5): DOI: 10.1242/dev.191320 PMID: 33674261.
2. *Nair N.* Epidemiology and pathogenesis of heart failure with preserved ejection fraction. Review. *Cardiovasc Med*. 2020; 21 (4): 531–540. DOI: 10.31083/j.rcm.2020.04.154.
3. *Mocumbi A.O., Stothard J.R., Correia-de-Sá P., Yacoub M.* Endomyocardial Fibrosis: an Update After 70 Years.*Curr Cardiol Rep*. 2019; 21 (11): 148. DOI: 10.1007/s11886-019-1244-3. PMID: 317583524.
4. *Zhou B., A. von Gise., Ma Q., Hu Y.W., Pu W.T.* Genetic fate mapping demonstrates contribution of epicardium-derived cells to the annulus fibrosis of the mammalian heart. *Dev. Biol*. 338 (2010) 251–261. DOI: 10.1016/j.ydbio.2009.12.007 PMID: 20025864. PMCID: PMC2815244
5. *Wessels. A, van den Hoff M.J., Adamo R.F., Phelps A.L., Lockhart M.M., Sauls K., Briggs L.E., Norris R.A., van Wijk B., Perez-Pomares J.M., Dettman R.W., Burch J.B.* Epicardially derived fibroblasts preferentially contribute to the parietal leaflets of the atrioventricular valves in the murine heart. *Dev. Biol*. 2012; 366: 111–124. DOI: 10.1016/j.ydbio.2012.04.020 PMID: 22546693. PMCID: PMC3358438
6. *von Gise A., Zhou B., Honor L.B., Ma Q., Petryk A., Pu W.T.* WT1 regulates epicardial epithelial to mesenchymal transition through beta-catenin and retinoic acid signaling pathways, *Dev. Biol*. 2011; 356: 421–431. DOI: 10.1016/j.ydbio.2011.05.668. PMID: 21663736 PMCID: PMC3147112
7. *Braitsch C.M., Combs M.D., Quaggin, S.E., Yutzey K.E.* Pod1/Tcf21 is regulated by retinoic acid signaling and inhibits differentiation of epicardium-derived cells into smooth muscle in the developing heart. *Dev. Biol*. 2012; 368: 345–357. DOI: 10.1016/j.ydbio.2012.06.002 PMID: 22687751. PMCID: PMC3414197
8. *Acharya, A., Baek, S.T., Huang, G., Eskiocak, B., Goetsch, S., Sung, C.Y., Banfi, S., Sauer M.F., OlsenG.S., Duffield J.S.* The bHLH transcription factor Tcf21 is required for lineage-specific EMT of cardiac fibroblast progenitors. *Development*. 2012; 139: 2139–2149. DOI: 10.1242/dev.079970. PMID: 22573622. PMCID: PMC3357908
9. *Moore-Morris T., Cattaneo P., Guimaraes-Camboa N., Bogomolovas J., Cedenilla M., Banerjee I., Ricote M., Kisseleva T., Zhang L., Gu Y., Dalton N.D., Peterson K.L., Chen J., Puceat M., Evans S.M.* Infarct fibroblasts do not derive from bone marrow lineages. *Circ. Res*. 2012; 122 (4): 583–590. DOI: 10.1161/CIRCRESAHA.117.311490. PMID: 29269349. PMCID: PMC5815911
10. *Moore-Morris T., Guimaraes-Camboa N., Banerjee I., Zambon A.C., Kisseleva T., Velayoudon A., Stallcup W.B., Gu Y. Dalton N.D, Cedenilla M., Gomez-Amaro R., Zhou B. Brenner D.A, Peterson K.L., Chen J., Evans S.M.* Resident fibroblast lineages mediate pressure overload-induced cardiac fibrosis. *J Clin Invest*. 2014; 124 (7): 2921–2934. DOI: 10.1172/JCI7478.3. PMID: 24937432. PMCID: PMC4071409
11. *Braitsch C.M., Kanisicak O., van Berlo J.H., Molkentin J.D, Yutzey K.E.* Differential expression of embryonic epicardial progenitor markers and localization of cardiac fibrosis in adult ischemic injury and hypertensive heart disease. *J. Mol. Cell. Cardiol*. 2013; 65: 108–119. DOI: 10.1016/j.yjmcc.2013.10.005. PMID: 24140724. PMCID: PMC3848425
12. *Santi A.Li., Napolitano F., Montuori N., Ragno P.* The Urokinase Receptor: A Multifunctional Receptor in Cancer Cell Biology. Therapeutic Implications. *Int J Mol Sci*. 2021; 22 (8): 4111. DOI: 10.3390/ijms22084111. PMID: 33923400. PMCID: PMC8073738
13. *Dergilev K.V., Stepanova V.V., Beloglazova I.B., Tsokolayev Z.I., Parfenova E.V.* Multifaced Roles of the Urokinase System in the Regulation of Stem Cell Niches. *Acta Naturae*. 2018; 10 (4): 19–32. PMID: 30713759. PMCID: PMC6351041
14. *Junqueira L.C., Bignolas G., Brentani R.R.* Picrosirius staining plus polarization microscopy, a specific method for collagen detection in tissue sections. *Histochem J*. 1979; 11: 447–455. DOI: 10.1007/bf01002772
15. *Dergilev K.V., Tsokolaeva Z.I., Beloglazova I.B., Ratner E.I., Parfenova E.V.* Transforming Growth Factor Beta (TGF-β1) Induces Pro-

Reparative Phenotypic Changes in Epicardial Cells in Mice.*Bull Exp Biol Med.* 2021; 170 (4): 565–570. DOI: 10.1007/s10517-021-05107-5.

16. *Baart V.M., Houvast R.D., de Geus-Oei L.F., Quax P.H.A., Kuppen P.J.K., Vahrmeijer A.L., Sier C.F.M.* Molecular imaging of the urokinase plasminogen activator receptor: opportunities beyond cancer. *EJNMMI Res.* 2020; 10 (1): 87. DOI: 10.1186/s13550-020-00673-7. PMID: 32725278. PMCID: PMC7387399
17. *Genua M., D'Alessio S., Cibella J., Gandelli A., Sala E., Correale C., Spinelli A., Arena V., Malesci A., Rutella S., Ploplis V.A., Vetrano S., Danese S.* The urokinase plasminogen activator receptor (uPAR) controls macrophage phagocytosis in intestinal inflammation. *Gut.* 2015; 64 (4): 589–600. DOI: 10.1136/gutjnl-2013-305933 PMID: 24848264
18. *Jo M., Takimoto S., Montel V., Gonias S.L.*The urokinase receptor promotes cancer metastasis independently of urokinase-type plasminogen activator in mice. *Am J Pathol.* 2009; 175 (1): 190–200. DOI: 10.2353/ajpath.2009.081053. PMID: 19497996. PMCID: PMC2708805
19. *Jo M., Lester R.D., Montel V., Eastman B., Takimoto S., Gonias S.L.* Reversibility of epithelial-mesenchymal transition (EMT) induced in breast cancer cells by activation of urokinase receptor-dependent cell signaling *J Biol Chem.* 2009; 284 (34): 22825–22833. DOI: 10.1074/jbc.M109.023960. PMID: 19546228 PMCID: PMC2755690
20. *Hinz B., Phan S.H., Thannickal V.J., Prunotto M., Desmoulière A., Varga J., De Wever O., Mareel M., Gabbiani G.* Recent developments in myofibroblast biology: paradigms for connective tissue remodeling. *Am J Pathol.* 2012; 180 (4): 1340–1355. DOI: 10.1016/j.ajpath. 2012.02.004. PMID: 22387320. PMCID: PMC3640252
21. *Castella L.F., Buscemi L., Godbout C., Meister J.J., Hinz B.* A new lock-step mechanism of matrix remodelling based on subcellular contractile events. *J Cell Sci.* 2010; 123 (Pt 10): 1751–1760. DOI: 10.1242/jcs.066795. PMID: 20427321
22. *Wang L., Ly C.M., Ko C.Y., Meyers E.E., Lawrence D.A., Bernstein A.M.* uPA binding to PAI-1 induces corneal myofibroblast differentiation on vitronectin. *Invest Ophthalmol Vis Sci.* 2012; 53 (8): 4765–4775. DOI: 10.1167/iovs.12-10042 PMID: 22700714. PMCID: PMC3949353
23. *Stepanova V., Lebedeva T., Kuo A., Yarovoi S., Tkachuk S., Zaitsev S., Bdeir K., Dumler I., Marks M.S., Parfyonova Y., Tkachuk V.A., Higazi A.A., Cines D.B.* Nuclear translocation of urokinase-type plasminogen activator. *Blood.* 2008; 112 (1): 100–110. DOI: 10.1182/blood-2007-07-104455. PMID: 18337556. PMCID: PMC2435680
24. *Semina E.V., Rubina K.A., Shmakova A.A., Rysenkova K.D., Klimovich P.S., Aleksanrushkina N. A., Sysoeva V. Y., Karagyaur M.N., Tkachuk V.A.* Downregulation of uPAR promotes urokinase translocation into the nucleus and epithelial to mesenchymal transition in neuroblastoma. *J Cell Physiol.* 2020; 235 (9): 6268–6286. DOI: 10.1002/jcp.29555. PMID: 31990070. PMCID: PMC7318179
25. *Zhang G., Kernan K.A., Thomas A., Collins S., Song Y., Li L., Zhu W., Leboeuf R.C., Eddy A.A.* A novel signaling pathway: fibroblast nicotinic receptor alpha1 binds urokinase and promotes renal fibrosis. *J Biol Chem.* 2009; 284 (42): 29050–29064. DOI: 10.1074/jbc.M109.010249. PMID: 19690163. PMCID: PMC2781451
26. *Manetti M., Rosa I., Fazi M., Guiducci S., Carmeliet P., Ibba-Manneschi L., Matucci-Cerinic M.* Systemic sclerosis-like histopathological features in the myocardium of uPAR-deficient mice. *Ann Rheum Dis.* 2016; 75 (2): 474–478. DOI: 10.1136/annrheumdis-2015-207803. PMID: 26269399
27. *Mohameden M., Vashisht P., Sharman T.* Scleroderma And Primary Myocardial Disease. 2021. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 Jan. PMID: 32491618
28. *Nikitorowicz-Buniak J., Denton C.P., Abraham D., Stratton R.* Partially Evoked Epithelial-Mesenchymal Transition (EMT) Is Associated with Increased TGFβ Signaling within Lesional Scleroderma Skin. *PLoS One.* 2015; 10 (7): e0134092. DOI: 10.1371/journal.pone.0134092. PMID: 26217927

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