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Svetlana I. Galkina, Galina F. Sud'ina, Ekaterina A. Golenkina, Tatyana G. Khryapenkova, Vladimir I. Stadnichuk, Evgenii A. Arifulin, Egor Y. Plotnikov, Dmitry B. Zorov

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Cytoneme-mediated cell clustering during rat renal epithelial monolayer formation and human neutrophil adhesion

Dear Editor,

While studying intercellular communication, reliable methods for isolating and cultivating renal tubular epithelial cells have been widely used^[1–2], but the mechanisms underlying the formation of homologous epithelial monolayers remain poorly understood. Using scanning electron microscopy, we explored the morphology of renal tubular epithelial cells during adhesion to the serum-coated substrate and monolayer formation. The cells were fixed after 12, 24, and 48 h after the initiation of culture. To our knowledge, we observed for the first time that from the initial stage of cell attachment to the substrate, when the cells were present in the form of globules consisting of round and partially flattened cells (**Fig. 1A**, a–h), until the formation of a flat monolayer (**Fig. 1A**, i), the cells were connected to each other (interconnected) by thin, long tubular filopodia. These filopodia are characterized by a strictly uniform diameter along the entire length, a high degree of flexibility, and the ability to reach exceptional lengths, which make them resemble fine flexible threads. We propose to use the term cytonemes (cytoplasmic threads), first proposed by Thomas Kornberg to describe specialized filopodia that mediate paracrine signaling in *Drosophila* embryonic cells^[3–4]. It should be noted that cytonemes in different clusters of renal epithelial cells may vary significantly in length (**Fig. 1A**, c and d).

Scanning electron microscopy does not allow determination of which cell the cytoneme emerges from and which cell it enters. Some cytonemes exited and entered the same cell. Other cytonemes extended into or emerged from distinct invaginations on the cell surface (**Fig. 1A**, e and f, blue arrowheads). Because of their well-defined boundaries, invaginations look like specialized cellular structures. The diameters of

cytonemes, invaginations, and microvilli were measured and averaged using ImageJ-win64 on scanning electron microscopy images (**Fig. 1B**). Our data showed that the average diameter of cytonemes in rat renal epithelial cells was 159 nm, which was significantly larger than the 121 nm diameter of microvilli (**Fig. 1C**).

Adhesion of renal epithelial cells to the substrate for 24 h resulted in the formation of significant areas of a monolayer of well-spread cells connected by cytonemes, which mainly disappeared after 48 h of incubation (**Fig. 1A**, i and j). The central location of cytonemes on the cell surface makes them resemble to non-motile primary cilia, specialized microtubule-based projections thought to function as cellular chemosensory and mechanosensory antennas. Mammalian renal epithelial cells contain a single non-motile primary cilium on the apical surface of the cell. The core of the primary cilium is the axoneme, which consists of nine microtubule doublets, a transition zone, and a basal body and is covered by a membrane "sheath" that is a continuation of the plasma membrane. The number of microtubule doublets in the axoneme and the diameter of the axoneme gradually decrease from the base to the tip of the cilium, since many microtubules do not reach their end. The diameter of the membrane-covered primary cilium of renal epithelial cells gradually decreases from approximately 300 nm at the base to about 200 nm at a distance of 3 μ m from the base to its distal end^[5]. Similar results were later obtained in islet cells, yielding an average base size of 237–240 nm and a tip size of 121–148 nm for primary cilia^[6]. In our experiments, cytonemes of renal epithelial cells were tubular structures with a uniform diameter of approximately 159 nm along their entire length. The cytoneme diameter was half the basal diameter of

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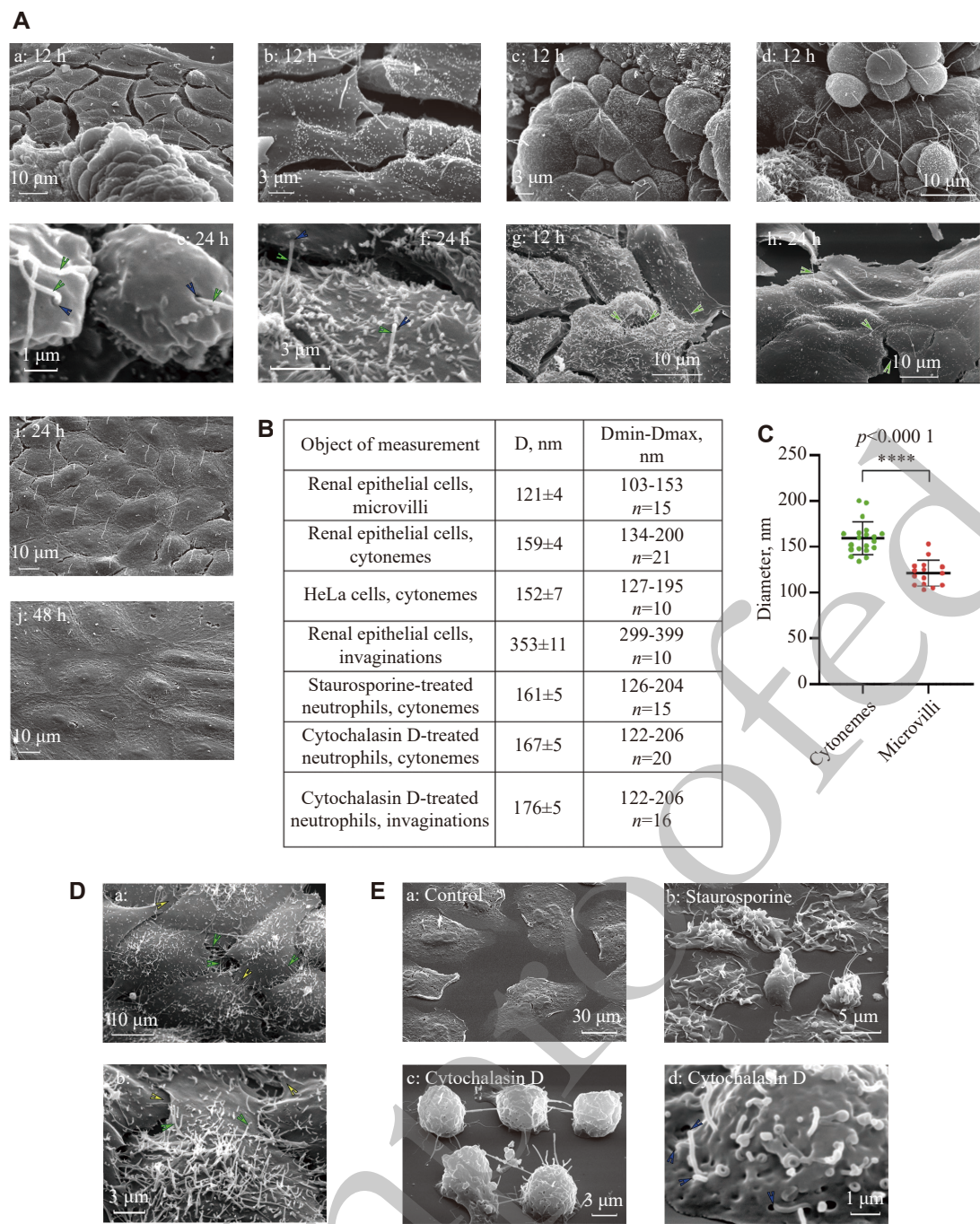


Fig. 1 Clustering of rat renal epithelial cells, HeLa cells, and human neutrophils by cytonemes. A: Scanning electron microscopy images of rat renal tubular epithelial cells attached to serum protein-coated coverslips for 12, 24 and 48 h. A cluster of renal epithelial cells adhered for 12 h, consisting of both flat (a and b) and globular fragments (c and d). The length of cytonemes connecting cells in different globules can vary significantly (c and d). Cytonemes and invaginations on the surface of round cells (e) and flat cells (f) that were attached to the substrate for 24 h. Blue arrowheads indicate invaginations, green arrowheads indicate cytonemes. Cytoneme tension (green arrowheads) during cell dissociation from original tissue and detachment (g and h). Monolayer of cells that were attached to the substrate for 24 h (i). Typical image of monolayer of cells that were attached for 48 h or longer (j). Representative images from three independent experiments are shown. B: The average diameter of the cytonemes, invaginations, and microvilli of rat renal epithelial cells, HeLa cells and human neutrophils. The data presented are the values of the diameters (mean ± standard deviation) that were obtained from the measurements of 10–20 cytonemes or invaginations on scanning electron microscopy images using ImageJ-win64 program. D_{min} and D_{max} are the observed minimal and maximal diameters. The results represent the average of three independent experiments. C: The average diameter of cytonemes and microvilli of rat renal epithelial cells was compared by unpaired Student's t -test. **** $P < 0.0001$. D: Scanning electron microscopy images of HeLa cell monolayer at different magnifications (a and b). Yellow arrowheads indicate lamellar, green arrowheads indicate filopodia (cytonemes). E: Scanning electron microscopy images of human neutrophils attached to fibronectin-coated coverslips. Neutrophils were allowed to adhere to fibronectin for 25 min under control conditions (a) or in the presence of 200 nmol/L staurosporine (b). Neutrophils were attached to fibronectin in the presence of 10 μmol/L cytochalasin D for 10 min (c) or 40 min (d). Blue arrowheads indicate cytonemes entering (exiting) distinct invaginations on the neutrophil bodies (d). Images are representative of the results of three independent experiments.

primary cilia (about 300 nm) (**Fig. 1A** and **1B**). Taken together, these observations indicate that cytonemes are structurally distinct from primary cilia.

Our images show that cytonemes connect cells, but this raises the question of whether cytonemes can physically hold cells in clusters under mechanical stress. Morphological changes in renal epithelial cells that enable the transformation of the original epithelium into a flat epithelial monolayer on a solid substrate include weakening of cell-cell contacts (**Fig. 1A**, g), apical-basal polarity, and interactions with the basement membrane (**Fig. 1A**, h). These changes provide cells with the motility necessary for dissociation from the original tissue, migrating, and attaching to the substrate. The apparent tension observed in cytonemes during cell dissociation (**Fig. 1A**, g and h, green arrows) may suggest the involvement of cytonemes in maintaining cells in clusters under mechanical stress.

Scanning electron microscopy of an immortal human epithelial cell line (HeLa) derived from a cervical cancer tumor (adenocarcinoma) revealed that the HeLa cell monolayer exhibited a characteristic feature of malignant cells, namely a lack of cell contact inhibition. The cells extended lamellae under and over neighboring cells (**Fig. 1D**, a and b, yellow arrowheads). The HeLa cell surface was covered with multiple filopodia—cytoneme-like protrusions of varying lengths that connected cells through gaps between cells (**Fig. 1D**, a and b, green arrowheads). They were almost identical in diameter to the cytonemes of renal tubular epithelial cells (**Fig. 1B**). Malignant HeLa cells, having lost many properties of epithelial cells, nevertheless retain the ability to form a monolayer of homogeneous cells. Cytonemes-mediated cell tethering could play a significant role in this process.

Cytonemes of rat renal epithelial cells also coincided in appearance and size with cytonemes of human neutrophils^[7–8]. Neutrophils were prepared from the fresh peripheral blood of healthy volunteers after obtaining written informed consent. Experiments were carried out following the ethical standards of the Helsinki Declaration. The experimental and the subject consent procedures were approved by the Bioethics Committee of the Lomonosov Moscow State University, Application # 6-h, version 3, Bioethics Commission meeting #131-d held on May 31, 2021. When neutrophils migrate and attach to the site of infection, they encounter actin-depolymerizing alkaloids released by invading microorganisms, such as staurosporine produced by *Streptomyces staurosporeus* or the mold alkaloid cytochalasin D^[9–11].

Neutrophils adhered to fibronectin for 25 min under control conditions were well spread but did not touch each other (**Fig. 1E**, a). Neutrophils adhered in the presence of staurosporine or cytochalasin D were formed interconnected cellular networks by long cytonemes (**Fig. 1E**, b and c), the diameter of which matched that of renal epithelial cytonemes (**Fig. 1B**). Cytonemes initiated by actin-depolymerizing agents are dynamic, highly flexible, and long structures, as shown in the phase-contrast microscopy video of cytonemes initiated by the sponge alkaloid latrunculin A, which induces actin depolymerization (**Supplementary Video 1**). Cytonemes induced by cytochalasin D were readily disrupted during preparation for electron microscopy. Electron microscopy images showed only cytoneme fragments, along with multiple invaginations with diameters similar to those of the cytonemes (**Fig. 1E**, c and d; **Fig. 1B**). Increasing the neutrophil adhesion time to 40 min or more resulted in further cell deformation, cytoneme disruption, and the formation of a series of invaginations of varying diameters, including invaginations similar in size (300–500 nm) to those observed on renal epithelial cells (**Fig. 1A**, e and f, blue arrowheads). Cytonemes on the surface of neutrophils penetrate (or exit) cells through these invaginations (**Fig. 1E**, d, blue arrowheads).

Cell tethering by cytonemes may represent a conserved biological phenomenon, as we have observed similarities in cytoneme morphology across mammalian species and cell types, such as rat renal epithelial cells, HeLa cells derived from human epithelial cells, and human neutrophils. The mechanism of cytoneme formation remains unclear. Neutrophils have been observed to form very long membrane cylinders, similar in diameter to cytonemes, during their flow over P-selectin-coated surfaces. The formation of membrane cylinders was explained by the binding of P-selectin glycoprotein ligand-1 (PSGL-1) receptors located at the tips of neutrophil microvilli to P-selectin and subsequent elongation of the microvilli under the action of fluid shear stress^[12]. These membrane structures can be extracted using a micropipette or optical capture tweezers and antibody-coated beads. Pretreatment of cells with the actin-depolymerizing alkaloids cytochalasin D or latrunculin A resulted in significant elongation of the membrane cylinders and an increase in their lifespan^[13].

In our experiments, extraction of cytonemes from neutrophils may have occurred due to the establishment and subsequent rupture of adhesive contacts between microvilli tips and adjacent cells

during the preparation of a flat monolayer. Fluid shear or mechanical stress during centrifugation and attachment to a solid substrate could have provided the tensile force for cytoneme extraction. The tensile force was apparently insufficient to extract the membrane cylinders from neutrophils under control conditions (**Fig. 1E**, a). Disruption of the cortical actin cytoskeleton with staurosporine, cytochalasin D, or latrunculin A could have promoted the formation of membrane cylinders, which were detected as cytonemes (**Fig. 1E**, b–d; **Supplementary Video 1**).

Cytoneme formation in renal epithelial cells may occur through a similar mechanism. Cytonemes may form as a result of the elongation of microvilli or membrane sheaths of primary cilia, the tips of which were attached to adjacent cells in the original tissue (**Fig. 1A**). Elongation of membrane tethers can occur under the influence of fluid shear or mechanical stress during cell preparation and attachment to a solid substrate to form a flat monolayer. Since the cytoneme diameter was significantly larger than the microvilli diameter (**Fig. 1C**), one possible explanation is that cytoneme formation occurs with elongation of the membrane sheaths of primary cilia. During the preparation process, rat epithelial cell clusters may experience various stresses, which may explain the development of cytonemes of varying lengths (**Fig. 1A**, c and d). We hypothesize that primary cilia and cytonemes are distinct but related structures that may play distinct but coordinated roles in cell physiology. Depolymerization of the actin cytoskeleton may contribute to cytoneme formation not only in neutrophils but also in renal epithelium. Literature data indicate that multicellular sheets of epithelial cells respond to mechanical stresses such as stretching, positioning at edges/curves of the epithelial layer, or stiffness of the extracellular matrix through the regulation of the transcriptional coactivator Yes-associated protein (YAP)/transcriptional coactivator with PDZ-binding motif (TAZ) by actin processing factors. F-actin-coating/severing proteins, cofilin, capZ, and gelsolin, limit YAP/TAZ activity in cells experiencing low mechanical stress^[14]. Recently, actin cytoskeleton reorganization has been recognized as a dominant inducer of ciliogenesis. Actin cytoskeleton polymerization, F-actin branching, and stress fiber formation all inhibit primary cilium formation, whereas actin depolymerization or depletion enhance ciliogenesis. Actin remodeling factors control ciliogenesis by regulating the transcriptional coactivator YAP/TAZ. Knockdown of YAP/TAZ is sufficient to induce ciliogenesis. Treatment with cytochalasin D or knockdown of LIMK2 (a serine-

threonine kinase known to inactivate cofilin and sensitive to inhibition by staurosporine) stimulates ciliogenesis in human retinal epithelial cells^[15].

In conclusion, to our knowledge, we provide the first ultrastructural evidence that renal epithelial cells after isolation, as well as during attachment to a solid substrate and monolayer formation, are connected to each other by long and flexible tubular processes or cytonemes. Cytoneme-mediated tethering of cells with flexible and easily elongated cytonemes may facilitate cluster migration and adhesion, potentially ensuring cell mobility and easy cluster reorganization under shear or mechanical stress. Cell aggregation and retention by cytonemes may help prevent cell dissociation and ensure a uniform epithelial monolayer. Cytoneme-mediated tethering of neutrophils could lead to neutrophil accumulation (swarming) at the site of infection, enhancing their bactericidal activity^[9,16].

Yours sincerely,

Author contributions

Galkina S.I.: Conceptualization, Methodology, Investigation, Writing —reviewing and editing. **Sud'ina G.F.:** Conceptualization, Methodology, Investigation, Funding acquisition, Writing. **Golenkina E.A.:** Methodology, Investigation, Formal analysis. **Khryapenkova T.G.:** Methodology, Investigation. **Stadnichuk V.I.:** Investigation, Software. **Arifulin E.A.:** Investigation, Visualization. **Plotnikov E.Y.:** Conceptualization, Methodology, Investigation, Resources. **Zorov D.B.:** Conceptualization, Methodology, Writing, Funding acquisition.

Additional information

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Yours sincerely,

Svetlana I. Galkina^{1,✉}, Galina F. Sud'ina¹, Ekaterina A. Golenkina¹, Tatyana G. Khryapenkova¹, Vladimir I. Stadnichuk², Evgenii A. Arifulin¹, Egor Y. Plotnikov¹, Dmitry B. Zorov^{1,✉}

¹A.N. Belozersky Institute of Physico-Chemical Biology, M.V. Lomonosov Moscow State University, Moscow 119991, Russia;

²Physical Department, M.V. Lomonosov Moscow

State University,
Moscow 119991, Russia.

✉Corresponding authors: Svetlana I. Galkina, E-mail: galkina@genebee.msu.ru, ORCID: 0000-0003-0196-2765; Dmitry Zorov, zorov@belozersky.msu.ru, ORCID: 0000-0003-0863-8490.

References

- [1] Plotnikov EY, Khryapenkova TG, Vasileva AK, et al. Cell-to-cell cross-talk between mesenchymal stem cells and cardiomyocytes in co-culture[J]. *J Cell Mol Med*, 2008, 12(5A): 1622–1631.
- [2] Plotnikov EY, Khryapenkova TG, Galkina SI, et al. Cytoplasm and organelle transfer between mesenchymal multipotent stromal cells and renal tubular cells in co-culture[J]. *Exp Cell Res*, 2010, 316(15): 2447–2455.
- [3] Ramirez-Weber FA, Kornberg TB. Cytonemes: Cellular processes that project to the principal signaling center in *Drosophila* imaginal discs[J]. *Cell*, 1999, 97(5): 599–607.
- [4] Wood BM, Baena V, Huang H, et al. Cytonemes with complex geometries and composition extend into invaginations of target cells[J]. *J Cell Biol*, 2021, 220(5): e202101116.
- [5] Sun S, Fisher RL, Bowser SS, et al. Three-dimensional architecture of epithelial primary cilia[J]. *Proc Natl Acad Sci U S A*, 2019, 116(19): 9370–9379.
- [6] Polino AJ, Sviben S, Melena I, et al. Scanning electron microscopy of human islet cilia[J]. *Proc Natl Acad Sci U S A*, 2023, 120(22): e2302624120.
- [7] Galkina SI, Fedorova NV, Serebryakova MV, et al. Proteome analysis identified human neutrophil membrane tubulovesicular extensions (cytonemes, membrane tethers) as bactericide trafficking[J]. *Biochim Biophys Acta*, 2012, 1820(11): 1705–1714.
- [8] Galkina SI, Fedorova NV, Golenkina EA, et al. Cytonemes versus neutrophil extracellular traps in the fight of neutrophils with microbes[J]. *Int J Mol Sci*, 2020, 21(2): 586.
- [9] Galkina SI, Stadnichuk VI, Molotkovsky JG, et al. Microbial alkaloid staurosporine induces formation of nanometer-wide membrane tubular extensions (cytonemes, membrane tethers) in human neutrophils[J]. *Cell Adh Migr*, 2010, 4(1): 32–38.
- [10] Galkina SI, Fedorova NV, Serebryakova MV, et al. Inhibition of the GTPase dynamin or actin depolymerisation initiates outward plasma membrane tubulation/vesiculation (cytoneme formation) in neutrophils[J]. *Biol Cell*, 2015, 107(5): 144–158.
- [11] Galkina SI, Fedorova NV, Serebryakova MV, et al. Mold alkaloid cytochalasin D modifies the morphology and secretion of fMLP-, LPS-, or PMA-stimulated neutrophils upon adhesion to fibronectin[J]. *Mediators Inflamm*, 2017, 2017: 4308684.
- [12] Schmidtke DW, Diamond SL. Direct observation of membrane tethers formed during neutrophil attachment to platelets or P-selectin under physiological flow[J]. *J Cell Biol*, 2000, 149(3): 719–730.
- [13] Marcus WD, Hochmuth RM. Experimental studies of membrane tethers formed from human neutrophils[J]. *Ann Biomed Eng*, 2002, 30(10): 1273–1280.
- [14] Aragona M, Panciera T, Manfrin A, et al. A mechanical checkpoint controls multicellular growth through YAP/TAZ regulation by actin-processing factors[J]. *Cell*, 2013, 154(5): 1047–1059.
- [15] Kim J, Jo H, Hong H, et al. Actin remodelling factors control ciliogenesis by regulating YAP/TAZ activity and vesicle trafficking[J]. *Nat Commun*, 2015, 6: 6781.
- [16] Golenkina EA, Galkina SI, Pletjushkina O, et al. Gram-negative bacteria *Salmonella typhimurium* boost leukotriene synthesis induced by chemoattractant fMLP to stimulate neutrophil swarming[J]. *Front Pharmacol*, 2022, 12: 814113.