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Transcriptomic and Histological Characterization of Telocytes in the Human Dorsal Root Ganglion

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ABSTRACT

Telocytes are interstitial cells characterized by long processes that span considerable distances within tissues, likely facilitating coordination and interaction with various cell types. Although present in central and peripheral neuronal tissues, their role remains elusive. Dorsal root ganglia (DRG) house pseudounipolar afferent neurons responsible for transmitting signals related to temperature, proprioception, and nociception. This study aimed to investigate the presence and function of telocytes in human DRG by examining their transcriptional profile, anatomical location, and ultrastructure.

Combined expression of *CD34* and *PDGFRA* is a marker gene set for telocytes, and our sequencing data revealed CD34 and PDGFRA expressing cells comprise roughly 1.5%–3% of DRG cells. Combined expression of *CD34* and *PDGFRA* is a putative marker gene set for telocytes. Further analysis identified nine subclusters with enriched cluster-specific genes. Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) pathway analysis suggested vascular, immune, and connective tissue-associated putative telocyte subtypes, mapping over 3000 potential receptor–ligand interactions between sensory neurons and these *CD34* and *PDGFRA* expressing putative telocytes were identified using a ligand–receptors interactome platform. Immunohistochemistry identified CD34+ve telocytes in the endoneural space of DRGs, next to neuron–satellite complexes, in perivascular spaces and in the endoneural space between nerve fiber bundles, consistent with pathway analysis. Transmission electron microscopy (TEM) confirmed their location identifying characteristic elongated nucleus, long and thin telopodes containing vesicles, often surrounded by a basal lamina. This study provides the first gene expression analysis of telocytes in complex human tissue, specifically the DRG, highlighting functional differences based on tissue location while revealing no significant ultrastructural variations.

1 | Introduction

Telocytes are interstitial, stromal cells found in connective tissues across many organs of the human body (see reviews Cretoiu and Popescu 2014; Kondo and Kaestner 2019). They possess a characteristic structure with long cellular processes (telopodes) that can show dilations (podomes) with segments between podomes termed podomers (Xiao and Bei 2016). Telocytes build

loose networks with connecting telopodes that can extend over 100 μm in length (Cretoiu and Popescu 2014) allowing connection with various other cell types (Cretoiu and Popescu 2014; Kondo and Kaestner 2019). They can be identified immunohistochemically with markers such as the hematopoietic stem cell marker CD34 or the platelet-derived growth factor (PDGF) receptor alpha (PDGFRA; Xiao and Bei 2016) although the immunohistochemical profile may vary across tissues (Diaz-Flores et al. 2020).

Telocytes have been described in many human organs (Kondo and Kaestner 2019) such as the gastrointestinal tract (GIT; Milia et al. 2013; Pieri et al. 2008), heart (Cretoiu et al. 2014; Gherghiceanu and Popescu 2012), lung (Manetti et al. 2014), urinary system (Vannucchi et al. 2014), skin (Manole et al. 2022), and the peripheral nervous system including enteric and parasympathetic ganglia (Diaz-Flores et al. 2020; Pieri et al. 2008; Rusu et al. 2016; Vandecasteele et al. 2017) and have been investigated in those organs under normal and pathological conditions including Crohn's disease (Milia et al. 2013), atherosclerosis (Xu et al. 2021), and urinary system pathologies (Sanches et al. 2021; Traini et al. 2018; Wishahi et al. 2021). However, the presence and ultrastructure of telocytes in human dorsal root ganglia (DRG) remain unknown. Recent transcriptomic studies in rodents have identified gene expression markers for telocytes which include *CD34*, *PDGFRA* and, to with less specificity, *DCN* (Guo et al. 2024) but gene expression in these cells has not been investigated at all in any human nervous system tissue.

DRG house the cell bodies of pseudounipolar sensory neurons responsible for transmitting nociception, temperature, itch, or mechanical stimulation signals (Haberberger et al. 2019). These sensory neurons form a complex with satellite glia cells (SGCs). Adjacent capillaries supply blood to meet energy demands, while Schwann cells envelop neuronal processes. Mammalian DRGs harbor several other cell types in addition to the neuron–satellite cell complex including macrophages, fibroblasts, and pericytes (Haberberger et al. 2019, 2023; Tavares-Ferreira et al. 2022). Recent studies explored the cell types in human DRGs via RNA-sequencing (RNA-seq) and subdivided the cells based on transcript enrichment into SGCs (Avraham et al. 2022), sensory neurons, and other non-neuronal cells (Bhuiyan et al. 2024; Jung et al. 2023; Nguyen et al. 2021; Tavares-Ferreira et al. 2022). Despite this, few studies have focused on analysis of non-neuronal cell types in human DRG (Avraham et al. 2022; Scaravilli et al. 1991). Using a whole cell RNA-seq technique we identified a subset of non-neuronal, nonglial cells that possess a transcriptional identity consistent with telocytes. We confirmed the presences of these cells in the human DRG based on immunohistochemical characteristics and ultrastructural morphology. Here, we propose that telocytes in the human DRG may play a key role in communication between the vasculature and neuron–glial units. In this study, we demonstrate the presence, transcriptional profile, and ultrastructure of telocytes in the human DRG, and propose functional roles for how these cells may interact with local sensory neurons, glial cells, and blood vessels within these ganglia.

2 | Material and Methods

2.1 | Human Tissue

All human tissue procurement procedures were approved by the Institutional Review Boards at the University of Texas at Dallas. Human lumbar DRGs were procured from three organ donors through a collaboration with the Southwest Transplant Alliance. DRGs were recovered using a ventral approach as previously described (Valtcheva et al. 2016). Donor medical history was provided by the Southwest Transplant Alliance and includes medical details from the donor's family and hospital records. Donor demographics, medical history, and DRG level details are provided in Supporting Information Table 1. For DRGs that were used for histological studies, upon removal from the body, the DRGs were cut in half, placed in 4% paraformaldehyde (PFA) for 48 h at 4°C, and then transferred to phosphate-buffered saline (PBS) and shipped on ice to the University of Adelaide in Australia for histological analyses. For DRGs used for RNA-seq methods are described below.

2.2 | Single-Cell RNA Sequencing

Single-cell RNA-seq from human DRGs was performed as previously described (dx.doi.org/10.17504/protocol.io.4r3I2qpr4I1y/v1; Hou et al. 2024). Human DRGs were surgically extracted from donors and placed in chilled, aerated artificial cerebrospinal fluid (aCSF). The aCSF solution contained 93 mM N-methyl-D-glucamine (NMDG; Sigma-Aldrich), HCl (12 N; Fisher), KCl (Sigma-Aldrich), NaH_2PO_4 (Sigma-Aldrich), $NaHCO_3$ (Sigma-Aldrich), HEPES (Sigma-Aldrich), D-(+)-glucose (Sigma-Aldrich), L-ascorbic acid (Sigma-Aldrich), thiourea (Sigma-Aldrich), Na+ pyruvate (Sigma-Aldrich), $MgSO_4$ (2 M; Fisher), $CaCl_2$ dihydrate (Sigma-Aldrich), and N-acetylcysteine (Sigma-Aldrich). The DRGs were transferred to a sterile petri dish on ice and trimmed to remove connective tissue and fat, using forceps and Bonn scissors. The dural coats (perineurium and epineurium) were carefully removed, isolating the ganglia bodies. These were further divided into approximately 1 mm thick sections using Bonn scissors. The tissue fragments were placed in 5 mL of a prewarmed enzyme mix containing Stemxyme 1, collagenase/neutral protease dispase, and deoxyribonuclease I (DNase I) in sterile filtered Hank's balanced salt solution (HBSS). The DRG–enzyme mixture was placed in a shaking water bath. It was gently triturated every 25 min using a sterile fire-polished glass Pasteur pipette until the solution turned cloudy and the tissue chunks passed smoothly through the pipette without resistance. Following enzymatic digestion, the dissociated DRGs were passed through a 100 μm cell strainer to remove debris and achieve a uniform cell suspension. The DRG cells were further isolated by layering the cell suspension on a 10% bovine serum albumin (BSA) solution prepared in sterile HBSS. The BSA gradient was then centrifuged at 300 × *g* for 10 min, resulting in the isolation of DRG cells. The supernatant was discarded, and the cell pellet was immediately fixed using the 10× Chromium Fixed RNA Profiling kit. The cells were fixed for 17 h at 4°C, followed by incubation with the 10× Fixed RNA Feature Barcode kit for 16 h. The

remainder of the library preparation was conducted according to the manufacturer's protocol. The samples were sequenced using a NextSeq2000 at the genome core facility at the University of Texas at Dallas. Sequencing data were processed and mapped to the human genome (GRCh38) using 10× Genomics Cell Ranger v7.

Data analysis for single-cell RNA-seq was conducted using the Seurat integration workflow (Stuart et al. 2019). To ensure data quality, only cells with less than 5% mitochondrial gene expression were included. The analysis workflow involved normalizing the data and selecting the top 2000 most variable features.

Following data scaling, standard clustering techniques using the Seurat pipeline (https://satijalab.org/seurat/get_started.html, Seurat (RRID:SCR_016341). The results were visualized using Uniform Manifold Approximation and Projection (UMAP) with a resolution parameter set to 1. UMAP a dimension reduction technique revealed presences of eleven distinct cluster populations in the human DRG. Next, we determined telocyte subsets from our single-cell database that met the conditions *CD34* > 1 and *PDGFRA* > 1. Cluster-specific markers were identified using the Wilcoxon rank-sum test implemented in Seurat and the telocyte marker gene list was ranked based on log2-fold change (log2FC).

Using a curated ligand–receptor database (Wangzhou et al. 2021) and the Sensoryomic web tool (https://sensoryomics.shinyapps.io/Interactome/), we conducted an interactome analysis. The initial analysis aimed to identify the top potential interactions between telocyte ligand gene markers and receptors expressed in human DRG neurons, based on expression data (Bhuiyan et al. 2024; Tavares-Ferreira et al. 2022). Subsequently, we explored potential interactions between telocyte receptor genes and ligand expressed in human DRG neurons. Ranking were determined by the log2FC values and a *p*-adjusted value of < 0.05 for telocyte-specific genes. Additionally, ligand and receptor genes were annotated with the protein class of their gene products using the PANTHER database (Thomas et al. 2022). The analysis focused on telocyte-receptor interactions with hDRG ligands and vice versa, identifying the top 50 receptor–ligand interactions.

The top 150 genes of each of the reclustered telocyte subclusters were analyzed by association with the Gene Ontology (GO) database (RRID:SCR_002811) and the Kyoto Encyclopedia of Genes and Genomes (KEGG; RRID:SCR_001120) using the online database STRING (http://STRING-db.org, RRID:SCR_005223). The database was also used for protein–protein network analysis.

2.3 | Multiple Labeling Immunofluorescence

Upon receipt from UTDallas, one half of an individual DRG was embedded in paraffin (Paraplast, Leica) using an automatic paraffin embedding system (HistoPearl, Leica). Sections (4 μM thickness) were cut, mounted on glass slides for hematoxylin and eosin (H&E) staining and on polyethylenimine (PEI)-coated slides for immunofluorescence (IF), dried overnight, deparaffinized using xylene and decreasing concentrations of ethanol and rehydrated. Sections were stained using routine H&E staining to evaluate the quality of the tissue and subsequently a second set of sections processed for multiple labeling IF.

For multiple labeling IF 10 mM citrate buffer pH 6 or Tris/EDTA pH 9 were used for antigen retrieval, followed by washing in PBS and multiple labeling immunohistochemistry (IHC). Before incubation with the primary antiserum or combination of antisera for 48 h at room temperature, unspecific binding sites were blocked using normal donkey serum. Incubation with the primary antisera was followed by washing in PBS and 2 h incubation with secondary antisera in combination with the nuclear stain 4′,6-diamidino-2-phenylindole (DAPI, Table 1). After an additional washing step in PBS, sections were covered with buffered glycerol pH 8.6, coverslipped, and sealed with nail varnish. A minimum of three technical replicates from each of the DRGs were investigated for each combination of antisera. The monoclonal CD34 antibody is directed against a human extracellular class II epitope. Binding specificity was checked by positive staining of Kaposi sarcoma. The CD31 antiserum is directed against the 130 kDA CD31 protein isolated from a membrane preparation of a patient with hairy cell leukemia. It was used in detection of blood vessels in human tissue including for the investigation of telocytes (Aleksandrovych et al. 2021; Yrigoin et al. 2024). The S-100 polyclonal antiserum is directed against purified S-100 protein from bovine brain and shows S-100 protein in human nervous system (Migheli et al. 1999). High-resolution images for entire sections of human DRG were acquired using a Slide Scanner (20× objective, Olympus VS200) and sections further investigated using confocal microscopy (Zeiss LSM 880 Fast Airyscan, Leica Mica).

2.4 | Electron Microscopy

The second half of the DRG was postfixed in 10% formalin for 48 h, washed in PBS and stored at 4°C until further processing. The tissues were subsequently cut into smaller pieces of about 1 mm^3 in size, fixed in 2.5% glutaraldehyde 2.5% in phosphate buffer, pH 7.4 at 4°C for 24 h, washed in PBS and transferred into 2% aqueous osmium tetroxide solution for 1 h. Then the samples were dehydrated in graded series of ethanol and embedded in epon araldite (TAAB) embedding medium at 60°C for 48 h. Semithin sections with a thickness of 500 nm were prepared and stained with 0.05% toluidine blue in borate buffer for 1 min. Ultrathin sections of 70–90 nm thickness were cut using a ultramicrotome (Leica), stained with 4% uranyl acetate and Reynolds lead citrate for 8 min and examined using an electron microscope (FEI Tecnai 120 kV Spirit). Images were captured using an AMT Camera with AMT_V7.0.1 software. Three to six sections from three different areas were investigated.

3 | Results

3.1 | In Silico Identification of *CD34* and *PDGFRA* Expressing Cells in Human DRG

Since it is known that telocytes express both *CD34* and *PDGFRA* we first sought to identify cells expressing both of these markers in a previously published harmonized atlas of DRG cell types. In this dataset of 34,693 non-neuronal single nuclei, we observed 767 cells that coexpressed *CD34* and *PDGFRA* (Bhuiyan et al. 2024). To further confirm this finding, we used a whole cell, single-cell RNA-seq dataset we have previously described that has the

TABLE 1 | Primary, secondary antibodies, and 4′,6-diamidino-2-phenylindole (DAPI).

Antigen	Host	Dilution	Supplier
CD34 (RRID:AB_2539474)	Mouse, clone QBEN10	1:600	Thermofisher
CD31 (RRID:AB_3099707)	Mouse, clone JC70A	1:500	Dako Cytomation
S100 (RRID:AB_477501)	Rabbit	1:2000	Sigma
DAPI		1:1000	Invitrogen
Antigen	**Host**	**Dilution**	**Supplier**
Mouse IgG-CY3 (RRID:AB_2315777)	Donkey	1:100	Jackson
Rabbit IgG-CY5 (RRID:AB_2340607)	Donkey	1:100	Jackson

advantage of being deeply sequenced and contains cytoplasmic mRNAs, giving a more thorough picture of gene expression for any given cell. Out of 41,678 cells sequenced, we identified 1287 cells that expressed both *CD34* and *PDGFRA*, making these putative telocytes (Figure 1A,B). We then generated a read count matrix for all 1287 cells creating a list of genes expressed in these cells on a per cell basis (Supporting Information Table 2). From this we reclustered the cells and identified nine subclusters that express both *CD34* and *PDGFRA* and we identified gene expression markers that were enriched in each cell population (Supporting Information Table 3). These nine subclusters of cells also expressed the telocyte marker *DCN* (Guo et al. 2024) further confirming their phenotype and strongly suggesting that there are multiple subtypes of telocytes in the human DRG (Figure 1C).

Next, we sought to understand potential interactions between telocytes and human DRG neurons using a ligand–receptors interactome platform (Wangzhou et al. 2021). Looking at ligands potentially produced by telocytes and their interactions with receptors in human DRG we identified 1621 interactions (Supporting Information Table 4) although many of these were for ligands that have multiple potential receptors. The top 50 ligand–receptor interactions, based on the expression level for the ligand and receptor pair, are shown in Figure 2A. The top interaction was the secreted WNT signaling factor *DKK2* from telocytes and the *LRP6* receptor which is expressed in many sensory neuron populations in the human DRG (Bhuiyan et al. 2024; Tavares-Ferreira et al. 2022). Another prominent pair was the *FGF18* gene, which encodes a secreted Fibroblast Growth Factor (FGF) family protein that can interact with many FGF receptors, in particular *FGFR1* that is highly expressed in human DRG neurons (Bhuiyan et al. 2024; Tavares-Ferreira et al. 2022). Three ligand–receptor pairs indicated a role of telocytes in the interplay between telocytes, neurons, and blood vessels. ANGPT1, TNFSF15, and THBS2 represent proteins with strong interaction with neuronal receptors but that have also been shown to maintain vascular structure (Huang et al. 2023; Saharinen and Alitalo 2011; Zhang and Li 2012). The telocyte ligand LSAMP belongs to the IgLON family of glycoproteins that are released and after extracellular processing integrated into the matrix of the neuronal cell surface (Kubick et al. 2018). It has possible interactions with a variety of neuronal receptors.

The interactome for ligands in human DRG neurons with receptors expressed by telocytes included 1830 interactions (Supporting Information Table 5) with the top 50 shown in Figure 2B. The top ligand–receptor interaction from sensory neurons to telocytes was the *PSAP* gene that encodes prosaponin and the receptor *CELSR1*, which is a member of the cadherin family. We also observed many

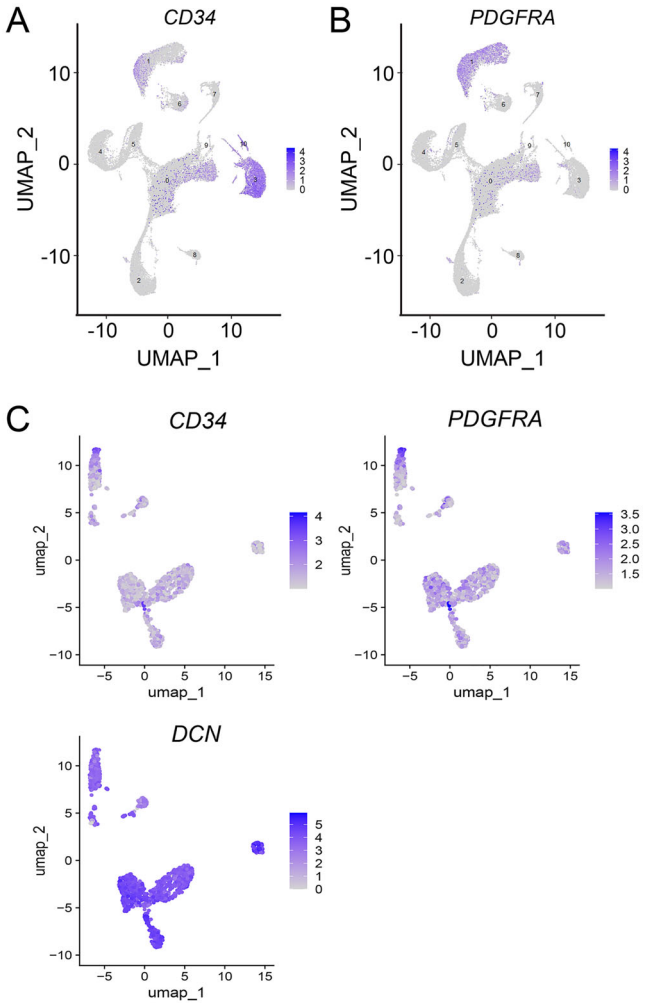

FIGURE 1 | Identification of telocytes in human dorsal root ganglia (DRG) using single-cell transcriptomics. (A, B) Uniform Manifold Approximation and Projection (UMAP) plots showing the expression of gene markers used to identify telocytes. (C) UMAP plots showing the expression of gene markers that reveal multiple telocyte clusters in the human DRG.

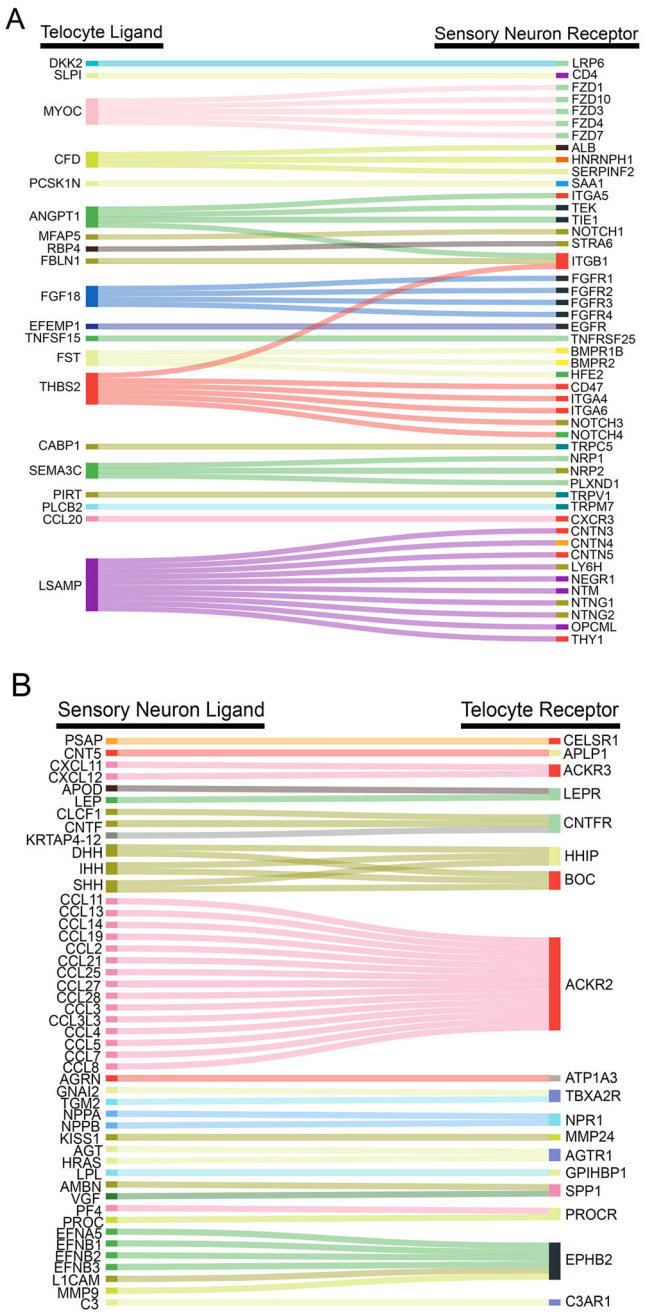

FIGURE 2 | Interactome analysis between neurons and telocytes in human dorsal root ganglia (DRG). (A) Sankey plot showing the top 50 unique telocyte-to-hDRG ligand–receptor interactions. (B) Sankey plot illustrating the top 50 hDRG-to-telocyte ligand–receptor interactions.

interactions with chemokines expressed by human DRG neurons and the atypical cytokine receptor type 2 (*ACKR2*) that binds promiscuously to many cytokines to limit their interactions with their cognate receptors. The CNTF receptor complex (*CNTFR*) is an additional cytokine receptor for the CNTF/LIF family of cytokines. CNTF is expressed in neurons and Schwann cells (Hu et al. 2020).

GO-enrichment analysis and KEGG-enrichment pathway analysis of the top 150 genes associated with telocyte subclusters showed that characteristic pathways are associated with individual subclusters. One of the nine subcluster showed that four out of six most enriched molecular functions (with a log10 (observed/expected)) were associated with extracellular matrix and collagen, whereas another telocyte subcluster showed half of the most enriched molecular functions were chemokine related, and the vast majority of enriched biological processes was associated with inflammation and immune system activation (Supporting Information Table 6). A third characteristic subcluster was associated with 21 (out of 32) strongly enriched biological processes that were related to blood vessels and/or vascular endothelium (Supporting Information Table 6).

3.2 | Multiple Labeling IF Reveals the Distribution of CD34+Ve Cells in Human DRG

To gain understanding into the distribution of telocytes in the DRG we used multiple labeling IF which revealed CD34-immunoreactivity in the endothelium of blood vessels, in single mononuclear cells in the interstitial space between neurons, and in cells with the morphology of telocytes with a spindle-shaped cell body and long telopodes surrounding the neuron–satellite complex (Figure 3). Telocytes were present close to the S100+ve satellite cell layer surrounding the somata of neurons but they did not extend their telopodes between satellite cells or onto the surface of neurons. The number of CD34+ve cells was high around neuron–satellite cell clusters and less prominent in areas with nerve fiber bundles (Figure 3). These CD34+ve telocytes were primarily present in the endoneurium and the space between endo- and perineurium in nerve fiber bundles and in the perivascular space around the microvasculature and small arterioles (Figure 3). The CD34+ telocytes were negative for CD31. This observation was in contrast with the CD34 immunoreactivity in the vascular endothelium that also showed immunoreactivity for CD31 (Figure 4).

3.3 | Transmission Electron Microscopy Outlines Ultrastructural Features of Telocytes in Human DRG

Next, to gain more detailed insights into the ultrastructural morphology of the human DRG, we used transmission electron microscopy (TEM) to confirm the presence of telocytes and their networks of telopodes. The presence small stellate- or spindle-shaped soma, an oval nucleus with intense heterochromatin and thin, and long telopodes were in line with observations of the characteristic telocyte features. The nuclei in DRG telocytes were elongated with dimensions of about 2–4 μm wide and 7–9 μm long and showed heterochromatin at their margins (Figures 5 and 6). These telocytes were typically bipolar or tripolar, occasionally featuring areas with slightly enlarged telopod diameters known as podomes (Figure 6), both containing vesicles and caveolae. Telocytes generally, but not always possessed a basal lamina, often accompanied by extracellular bundles of collagen fibers (Figures 5, 6 and Supporting Information Figures 1, 2).

Telocytes were located adjacent to the nerve cell/satellite glia complex (Figure 5) but were also present in the internal perineurium as well as the endoneurium of nerve fiber bundles and at the outer border of the perivascular space around small blood

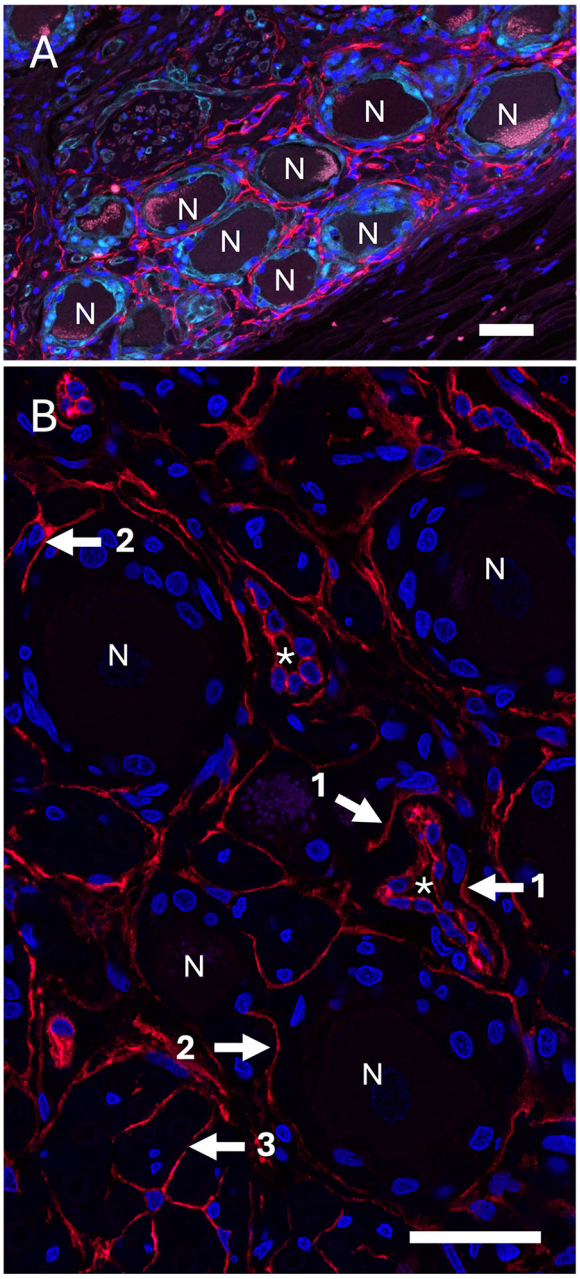

FIGURE 3 | Multiple-labeling immunofluorescence (IF) for CD34 (red), 4′,6-diamidino-2-phenylindole (DAPI) (blue) (A, B) and S100 (cyan) (A). CD34 immunoreactivity is present around neuron (N)–satellite glia complexes, adjacent to S100+ve satellite cells (A). The confocal image (B) shows CD34+ve cells around blood vessels (1), surrounding nerve cell/satellite glia complexes (2) and in the endoneural space (3). Blood vessels also showed CD34 immunoreactivity (asterisks). Bars = 50 μm.

vessels (Figure 6). Interestingly, the vessel-associated telocytes were always bordering the perivascular space (Figure 6).

Telopodes of perivascular telocytes and telocytes spanning the other parts of the endoneural space were found running parallel to each other without cell–cell contacts. Occasionally electron-dense cell–cell contacts between telopodes were observed (Figure 6 and Supporting Information Figures 1, 2) but no cell–cell contacts of telocytes with SGCs or neurons could be identified. Telopodes of telocytes that surround small blood

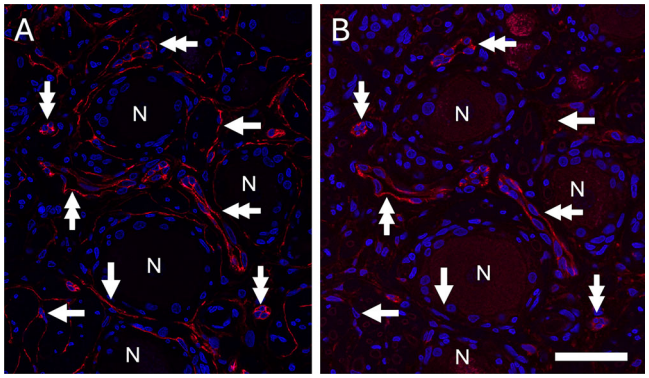

FIGURE 4 | Immunofluorescence for CD34 (A) and CD31 (B) in combination with 4′,6-diamidino-2-phenylindole (DAPI) in consecutive sections. The double arrowheads indicate the CD34+ve (A) and CD31+ve (B) vascular endothelium of the microvasculature. Arrows show CD34+ve telocytes (A) that do not express immunoreactivity for CD31 (B) close to the neuron (N)–satellite complex. Bar = 50 μm.

vessels were usually shorter compared to the extended processes of telocytes that surround nerve fiber bundles or traversed the remaining endoneural space.

4 | Discussion

Most investigations on DRG focus on neurons but the ganglia are comprised various cell types including satellite cells or endothelial cells which outnumber neurons. However, the identification of cell types in human DRG is primarily based on recent transcriptional studies, which might exclude or underrepresent certain cell types. One cell type that has thus far not been investigated are telocytes.

4.1 | Marker Genes and Expression Patterns Define Nine Subclusters of Telocytes

In this study, we used two previously published single nucleus (Bhuiyan et al. 2024) and single-cell RNA-seq datasets (Hou et al. 2024) to identify putative telocytes in the human DRG. Telocytes were identified using the marker genes *CD34* and *PDGFRA*, with *DCN* used as an additional coexpression marker that is less specific for telocytes (Guo et al. 2024). Our analysis revealed that these cells constitute approximately 1.5%–3% of all human DRG cells in these datasets.

Reclustering of these cells from the single-cell sequencing dataset, which captures cytoplasmic mRNAs giving a more comprehensive view of gene expression in these cells, suggests the presence of several subtypes of telocytes in the DRG. This is supported by studies on the murine intestine where different telocyte subpopulations have been described (reviewed by Shoshkes-Carmel 2024). Our analysis identified nine clusters and subsequent GO-enrichment analysis and KEGG-enrichment pathway analysis revealed differential gene and signaling pathway enrichments among these clusters. Each cluster contained specific enrichments in genes and signaling pathways including those associated with immune system, vasculature, or connective

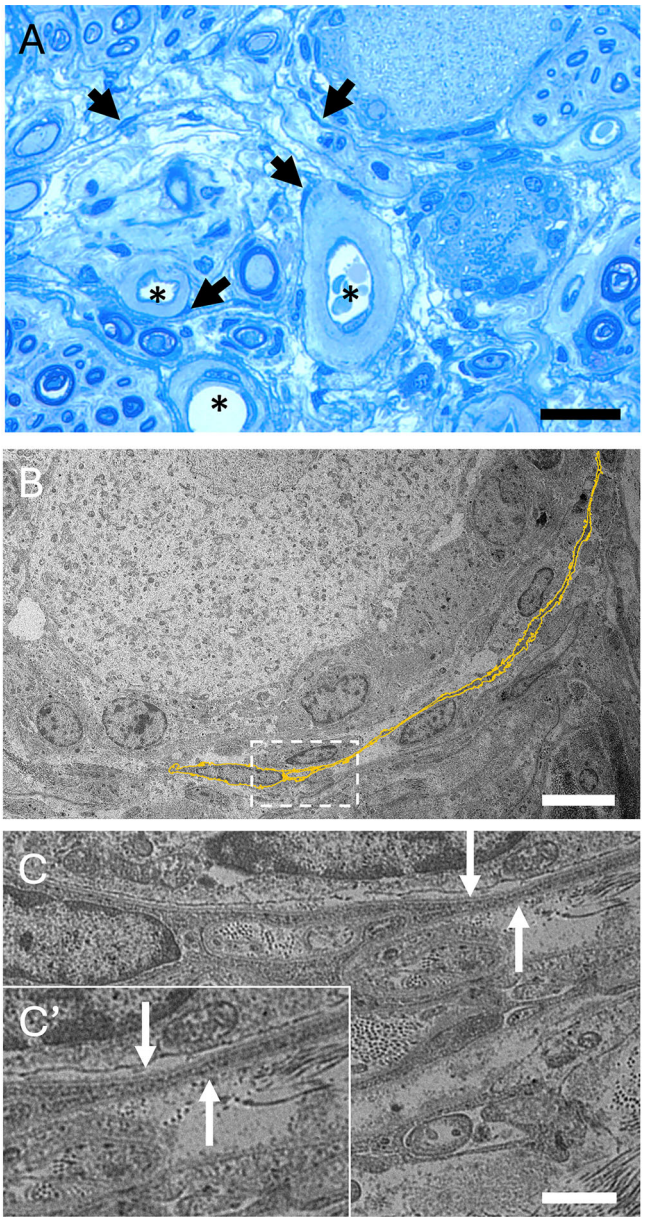

FIGURE 5 | The brightfield image of a toluidine blue stained semithin section (A) shows neuronal somata, satellite cells, nerve fiber bundles, and blood vessels. Telocyte nuclei next to the neuron–satellite complex, in the perivascular and interstitial space are indicated with black arrows, blood vessels with asterisks. Bar = 20 μm. The transmission electron microscopy (TEM) image (B) shows a telocyte at the nerve cell/satellite glia complex border. Cell body and telopode are pseudocolored. Bar = 6 μm. The dashed box in B indicates the area shown in C and C′. Images C and C′ show a higher magnification of the telopode. The cell membrane and adjacent basal lamina are indicated with arrows. Bar = 1 μm.

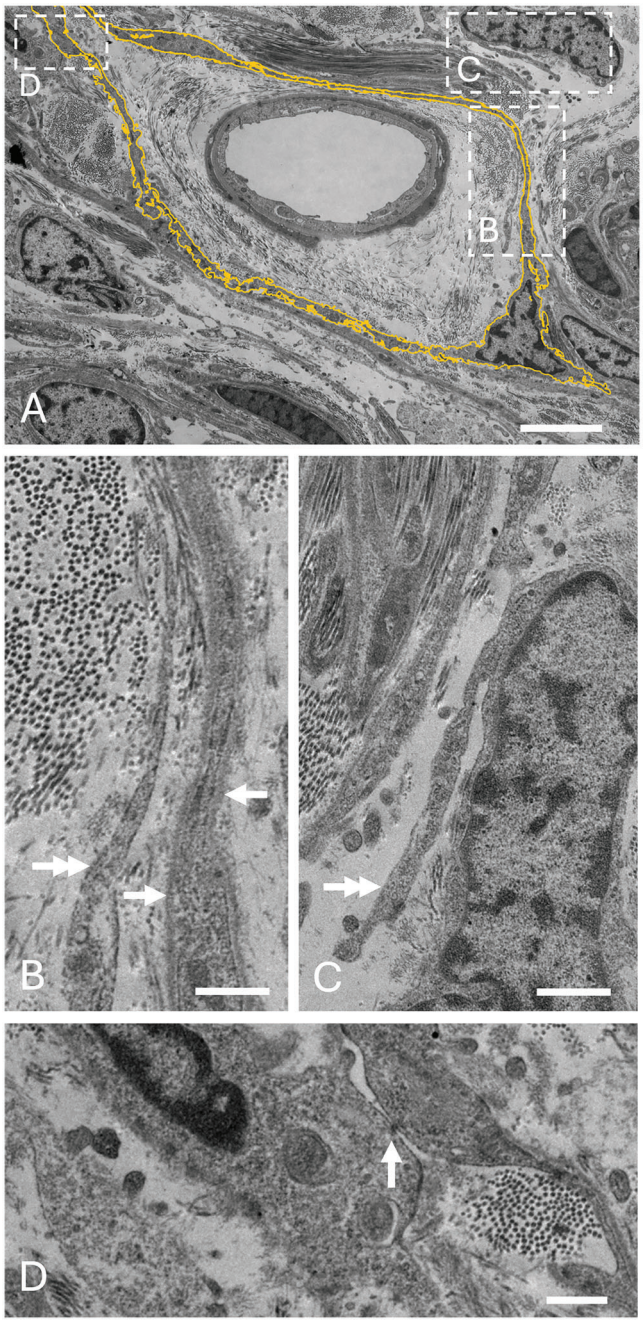

FIGURE 6 | The transmission electron microscopy (TEM) image (A) shows a telocyte surrounding a small blood vessel and the perivascular space. The telopodes completely surround the space. Cell body and telopodes are pseudocolored. Bar = 4 μm. The dashed boxes in A indicate the areas shown in B and C and D. The image B shows the presence of a basal lamina at the telopode (arrows) whereas a neighboring cell process (double arrowhead) does not possess a basal lamina. Bar = 1 μm. C shows a neighboring cell with a process (double arrowhead) that does not possess a basal lamina. Bar = 1 μm. The image D shows an electron-dense contact between a podome-like elongation with another cell. Bar = 1 μm.

tissue. Analysis of possible communication between telocytes and neurons revealed numerous potential ligand–receptor interactions between telocytes and sensory neurons, and vice versa. Notably, the expression of atypical cytokine receptor (*ACKR2*; Bonavita et al. 2016) in telocytes could limit the local action of chemokines released by DRG neurons.

4.2 | Location and Ultrastructure of Telocytes

Our analysis of sequencing data indicates the presence of telocytes in human DRG and suggests their potential involvement in diverse functions. Given that cellular location is often correlated with function, we further investigated the spatial distribution

and ultrastructure of telocytes within human DRG. Previous studies have reliably identified cells expressing CD34 mRNA in human DRG using immunohistochemical techniques with antisera against the CD34 protein. Therefore, we employed this established marker to localize and characterize telocytes in our samples (Diaz-Flores et al. 2023; Rosa et al. 2023; Sanches et al. 2023). This study identified CD34 immunoreactive endothelial cells in microvessels without a muscularis layer, likely capillaries or small postcapillary venules. The strong immunoreactivity confined to the endothelium is consistent with previous studies in human tissue (Fina et al. 1990; Pusztaszeri et al. 2006; Rusu et al. 2018). Additionally, immunoreactivity for CD34 was present in elongated cells with distinct telocyte-like morphology such as long and thin telomere-like processes. IF analysis revealed CD34-positive cells within the endoneural space, specifically adjacent to blood vessels, neuron–satellite cell complexes, and interspersed between nerve fiber bundles. The observed location, morphology, and CD34 expression of these cells are consistent with the characteristics of telocytes, as described in other human tissues (Diaz-Flores et al. 2020; Rusu et al. 2016; Traini et al. 2018).

Using TEM to better understand the characteristic morphology of human DRG telocytes, revealed the characteristic ultrastructure including the elongated ovoid nucleus, telopodes, and podomes (Cretoiu and Popescu 2014; Diaz-Flores et al. 2020; Xiao and Bei 2016). The size of telocyte cell bodies was consistent with measurements reported in the literature (see review Wang et al. 2016). Telocytes were identified bordering the perivascular space, surrounding microvessels, and near neuron–satellite complexes in human DRG similar to their distribution pattern in the human trigeminal ganglion which shares morphological and transcriptional features with the DRG (Bhuiyan et al. 2023; Rusu et al. 2016). The cell bodies and telopodes of telocytes next to neuron–satellite complexes and of telocytes bordering the perivascular space were often, but not always, surrounded by a basal lamina. Telocytes were localized in close proximity to neuron–satellite complexes but we did not observe direct connections to cell bodies or processes of sensory neurons, nor did we find conclusive evidence of any connections with satellite cells. Nonetheless, the regular presence of telocytes in at least three different locations supports our expression analysis data which suggest the presence of different telocyte subtypes or functional states.

4.3 | Connection of Expression and Location to Function

Although this study provides critical insights into the complexity of telocyte biology in the human DRG, however, the precise role of telocytes in human DRG remains unclear. Insights into human telocyte function have been mostly derived from studies using tissue samples from patients. Although functional data on telocytes in humans are limited (Albulescu et al. 2015; Liao et al. 2021; Manole et al. 2011; Sanches et al. 2023), descriptive studies showed that the number and localization of telocytes is altered in different diseases such as atherosclerosis, megaureter, or dermal fibrosis (Diaz-Flores et al. 2020; Rosa et al. 2021; Smith et al. 2023; Wishahi et al. 2021; Xu et al. 2021). In this study we identified telocytes throughout the endoneural connective tissue space forming a three-dimensional network in the human DRG. This network may be considered as part of the stroma, providing structural support to the surrounding tissue, given that stromal-cell-derived factor-1 has been shown to promote telocyte proliferation (Sanches et al. 2023). However, telocytes might play a more active role in tissue function beyond structural support, especially since the ultrastructure of these cells characterized by abundance of vesicles and occasional multivesicular bodies involved in cell–cell signaling (Cretoiu and Popescu 2014; Ratajczak et al. 2016) and clearly supported by the expression analysis findings of this study.

Indeed, telocytes from other tissues are capable of releasing vesicles into the extracellular environment (Fertig et al. 2014) which interact with neighboring cells (Cismasiu and Popescu 2015). For instance, cardiac telocytes have been found to transfer vesicles loaded with microRNAs (miRNA) to local stem cells, and then reacquire the repackaged miRNAs via vesicular endocytosis from the same stem cells (Cismasiu and Popescu 2015). Given the high density of vesicles and multivesicular cargos within human DRG telocytes, it can be hypothesized that similar bidirectional signaling mechanisms might be present between DRG telocytes and cells in close proximity, likely the satellite glia and sensory neurons. Our sequencing data support that human DRG telocytes are abundant in ligands that can interact with receptors found on satellite glial cells and sensory neurons. One miRNA that has been shown to be released from telocytes is miR-21, which infers a protective function on DRG neurons but also contributes to neuropathic pain (Buller et al. 2010; Zhou et al. 2015).

Telocytes and their processes surround endothelial cells and adjacent pericytes of small blood vessels which is corroborated by studies in human heart, gut, and parathyroid glands (Diaz-Flores et al. 2022; Liao et al. 2021; Smith et al. 2023). This indicates a role of telocytes related to blood vessel function. It is supported by our analysis of sequencing data which showed a high number of vasculature-related genes in one of the telocyte clusters. Capillaries and venules in DRGs are located near neurons–satellite cell complexes that have a high energy demand. The expression of angiopoietin 1 (*ANGPT1*), thrombospondin 2, and TNFSF15 suggests interactions between telocytes, blood vessels, and neurons. Angiopoietin 1 is important for the maintenance of vascular structure and vessel permeability, thrombospondin 2 (*THBS2*) is a matricellular protein that inhibits angiogenesis and tumor necrosis factor superfamily-15 (*TNFSF15*) protein is a cytokine that is known as vascular endothelial growth inhibitor which also inhibits endothelial cell differentiation and proliferation (Bornstein 2009; Brindle et al. 2006; Zhang and Li 2012).

4.4 | Summary and Outlook

In summary, our analysis of gene expression patterns in telocytes suggests, for the first time, the existence of distinct telocyte subpopulations within human DRG. The unique gene expression profiles not only link to specific cellular functions but also shed light on the multifaceted roles of telocytes in the DRG microenvironment. Future investigations will provide important confirmation in relation to individual pathways and biological processes or conditions related to telocyte subtypes. Our division telocytes into multiple phenotypes are further supported by multiple-labeling immunohistochemistry and ultrastructural

investigations, which underscore the potential for critical and diverse functions that human DRG telocytes may possess. This discovery opens new avenues for understanding DRG physiology and potentially offers novel targets for therapeutic interventions in conditions with DRG dysfunction.

Author Contributions

Rainer V. Haberberger designed, conducted, and analyzed the immunohistological and electron microscopical experiments, prepared the manuscript and figures. **Dusan Matusica** prepared and edited the manuscript and final figures. **Stephanie Shiers** dissected the human DRG, performed image analysis, and edited the manuscript. **Ishwarya Sankaranarayanan** conducted the in silico analyses, prepared, and edited the manuscript. **Theodore J. Price** oversaw the study, codesigned the experiments, prepared, and edited the manuscript.

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Ethics Statement

All human tissue procurement procedures were approved by the Institutional Review Boards at the University of Texas at Dallas. Human lumbar DRGs were procured from three organ donors through a collaboration with the Southwest Transplant Alliance. The use of human tissue was approved by the Office of Research Ethics, Compliance and Integrity, The University of Adelaide.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in SPARC at https://mapcore-demo.org/staging/sparc-app/datasets/407?type=dataset, reference number 10.26275/pxwy-sric.

Peer Review

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References

Albulescu, R., C. Tanase, E. Codrici, D. I. Popescu, S. M. Cretoiu, and L. M. Popescu. 2015. "The Secretome of Myocardial Telocytes Modulates the Activity of Cardiac Stem Cells." *Journal of Cellular and Molecular Medicine* 19, no. 8: 1783–1794. https://doi.org/10.1111/jcmm.12624.

Aleksandrovych, V., A. Wrona, T. Bereza, K. Pitynski, and K. Gil. 2021. "Oviductal Telocytes in Patients With Uterine Myoma." *Biomedicines* 9, no. 8: 1060. https://doi.org/10.3390/biomedicines9081060.

Avraham, O., A. Chamessian, R. Feng, et al. 2022. "Profiling the Molecular Signature of Satellite Glial Cells at the Single Cell Level Reveals High Similarities Between Rodents and Humans." *Pain* 163, no. 12: 2348–2364. https://doi.org/10.1097/j.pain.0000000000002628.

Bhuiyan, S. A., M. Xu, L. Yang, et al. 2023. "Harmonized Cross-Species Cell Atlases of Trigeminal and Dorsal Root Ganglia." *BioRxiv*. https://doi.org/10.1101/2023.07.04.547740.

Bhuiyan, S. A., M. Xu, L. Yang, et al. 2024. "Harmonized Cross-Species Cell Atlases of Trigeminal and Dorsal Root Ganglia." *Science Advances* 10, no. 25: eadj9173. https://doi.org/10.1126/sciadv.adj9173.

Bonavita, O., V. Mollica Poeta, E. Setten, M. Massara, and R. Bonecchi. 2016. "ACKR2: An Atypical Chemokine Receptor Regulating Lymphatic Biology." *Frontiers in Immunology* 7: 691. https://doi.org/10.3389/fimmu.2016.00691.

Bornstein, P. 2009. "Thrombospondins Function as Regulators of Angiogenesis." *Journal of Cell Communication and Signaling* 3, no. 3–4: 189–200. https://doi.org/10.1007/s12079-009-0060-8.

Brindle, N. P., P. Saharinen, and K. Alitalo. 2006. "Signaling and Functions of Angiopoietin-1 in Vascular Protection." *Circulation Research* 98, no. 8: 1014–1023. https://doi.org/10.1161/01.RES.0000218275.54089.12.

Buller, B., X. Liu, X. Wang, et al. 2010. "MicroRNA-21 Protects Neurons From Ischemic Death." *FEBS Journal* 277, no. 20: 4299–4307. https://doi.org/10.1111/j.1742-4658.2010.07818.x.

Cismasiu, V. B., and L. M. Popescu. 2015. "Telocytes Transfer Extracellular Vesicles Loaded With microRNAs to Stem Cells." *Journal of Cellular and Molecular Medicine* 19, no. 2: 351–358. https://doi.org/10.1111/jcmm.12529.

Cretoiu, D., E. Hummel, H. Zimmermann, M. Gherghiceanu, and L. M. Popescu. 2014. "Human Cardiac Telocytes: 3D Imaging by FIB-SEM Tomography." *Journal of Cellular and Molecular Medicine* 18, no. 11: 2157–2164. https://doi.org/10.1111/jcmm.12468.

Cretoiu, S. M., and L. M. Popescu. 2014. "Telocytes Revisited." *Biomolecular Concepts* 5, no. 5: 353–369. https://doi.org/10.1515/bmc-2014-0029.

Diaz-Flores, L., R. Gutierrez, M. P. Garcia, et al. 2020. "Telocytes in the Normal and Pathological Peripheral Nervous System." *International Journal of Molecular Sciences* 21, no. 12: 4320. https://doi.org/10.3390/ijms21124320.

Diaz-Flores, L., R. Gutierrez, M. P. Garcia, et al. 2022. "Comparison of the Behavior of Perivascular Cells (Pericytes and CD34+ Stromal Cell/Telocytes) in Sprouting and Intussusceptive Angiogenesis." *International Journal of Molecular Sciences* 23, no. 16: 9010. https://doi.org/10.3390/ijms23169010.

Diaz-Flores, L., R. Gutierrez, M. Gonzalez-Gomez, et al. 2023. "Telocytes/CD34+ Stromal Cells in the Normal, Hyperplastic, and Adenomatous Human Parathyroid Glands." *International Journal of Molecular Sciences* 24, no. 15: 2118. https://doi.org/10.3390/ijms241512118.

Fertig, E. T., M. Gherghiceanu, and L. M. Popescu. 2014. "Extracellular Vesicles Release by Cardiac Telocytes: Electron Microscopy and Electron Tomography." *Journal of Cellular and Molecular Medicine* 18, no. 10: 1938–1943. https://doi.org/10.1111/jcmm.12436.

Fina, L., H. V. Molgaard, D. Robertson, et al. 1990. "Expression of the CD34 Gene in Vascular Endothelial Cells." *Blood* 75, no. 12: 2417–2426.

Gherghiceanu, M., and L. M. Popescu. 2012. "Cardiac Telocytes—Their Junctions and Functional Implications." *Cell and Tissue Research* 348, no. 2: 265–279. https://doi.org/10.1007/s00441-012-1333-8.

Guo, W., Z. Zhang, J. Kang, Y. Gao, P. Qian, and G. Xie. 2024. "Single-Cell Transcriptome Profiling Highlights the Importance of Telocyte, Kallikrein Genes, and Alternative Splicing in Mouse Testes Aging." *Scientific Reports* 14, no. 1: 14795. https://doi.org/10.1038/s41598-024-65710-0.

Haberberger, R. V., C. Barry, N. Dominguez, and D. Matusica. 2019. "Human Dorsal Root Ganglia." *Frontiers in Cellular Neuroscience* 13: 271. https://doi.org/10.3389/fncel.2019.00271.

Haberberger, R. V., J. Kuramatilake, C. M. Barry, and D. Matusica. 2023. "Ultrastructure of Dorsal Root Ganglia." *Cell and Tissue Research* 393, no. 1: 17–36. https://doi.org/10.1007/s00441-023-03770-w.

Hou, J., Y. Chen, Z. Cai, et al. 2024. "Antibody-Mediated Targeting of Human Microglial Leukocyte Ig-Like Receptor B4 Attenuates Amyloid Pathology in a Mouse Model." *Science Translational Medicine* 16, no. 741: eadj9052. https://doi.org/10.1126/scitranslmed.adj9052.

Hu, Z., N. Deng, K. Liu, N. Zhou, Y. Sun, and W. Zeng. 2020. "CNTF-STAT3-IL-6 Axis Mediates Neuroinflammatory Cascade Across Schwann Cell-Neuron-Microglia." *Cell Reports* 31, no. 7: 107657. https://doi.org/10.1016/j.celrep.2020.107657.

Huang, J., C. Wang, Y. Hou, et al. 2023. "Molecular Mechanisms of Thrombospondin-2 Modulates Tumor Vasculogenic Mimicry by PI3K/AKT/mTOR Signaling Pathway." *Biomedicine & Pharmacotherapy* 167: 115455. https://doi.org/10.1016/j.biopha.2023.115455.

Jung, M., M. Dourado, J. Maksymetz, et al. 2023. "Cross-Species Transcriptomic Atlas of Dorsal Root Ganglia Reveals Species-Specific Programs for Sensory Function." *Nature Communications* 14, no. 1: 366. https://doi.org/10.1038/s41467-023-36014-0.

Kondo, A., and K. H. Kaestner. 2019. "Emerging Diverse Roles of Telocytes." *Development (Cambridge, England)* 146, no. 14: dev175018. https://doi.org/10.1242/dev.175018.

Kubick, N., D. Brosamle, and M. E. Mickael. 2018. "Molecular Evolution and Functional Divergence of the IgLON Family." *Evolutionary Bioinformatics Online* 14: 1176934318775081. https://doi.org/10.1177/1176934318775081.

Liao, Z., Y. Chen, C. Duan, et al. 2021. "Cardiac Telocytes Inhibit Cardiac Microvascular Endothelial Cell Apoptosis Through Exosomal miRNA-21-5p-Targeted cdip1 Silencing to Improve Angiogenesis Following Myocardial Infarction." *Theranostics* 11, no. 1: 268–291. https://doi.org/10.7150/thno.47021.

Manetti, M., I. Rosa, L. Messerini, S. Guiducci, M. Matucci-Cerinic, and L. Ibba-Manneschi. 2014. "A Loss of Telocytes Accompanies Fibrosis of Multiple Organs in Systemic Sclerosis." *Journal of Cellular and Molecular Medicine* 18, no. 2: 253–262. https://doi.org/10.1111/jcmm.12228.

Manole, C. G., V. Cismasiu, M. Gherghiceanu, and L. M. Popescu. 2011. "Experimental Acute Myocardial Infarction: Telocytes Involvement in Neo-Angiogenesis." *Journal of Cellular and Molecular Medicine* 15, no. 11: 2284–2296. https://doi.org/10.1111/j.1582-4934.2011.01449.x.

Manole, C. G., M. Gherghiceanu, L. C. Ceafalan, and M. E. Hinescu. 2022. "Dermal Telocytes: A Different Viewpoint of Skin Repairing and Regeneration." *Cells* 11, no. 23: 3903. https://doi.org/10.3390/cells11233903.

Migheli, A., S. Cordera, C. Bendotti, C. Atzori, R. Piva, and D. Schiffer. 1999. "S-100beta Protein Is Upregulated in Astrocytes and Motor Neurons in the Spinal Cord of Patients With Amyotrophic Lateral Sclerosis." *Neuroscience Letters* 261, no. 1–2: 25–28. https://doi.org/10.1016/s0304-3940(98)01001-5.

Milia, A. F., M. Ruffo, M. Manetti, et al. 2013. "Telocytes in Crohn's Disease." *Journal of Cellular and Molecular Medicine* 17, no. 12: 1525–1536. https://doi.org/10.1111/jcmm.12177.

Nguyen, M. Q., L. J. von Buchholtz, A. N. Reker, N. J. Ryba, and S. Davidson. 2021. "Single-Nucleus Transcriptomic Analysis of human Dorsal Root Ganglion Neurons." *Elife* 10: e71752. https://doi.org/10.7554/eLife.71752.

Pieri, L., M. G. Vannucchi, and M. S. Faussone-Pellegrini. 2008. "Histochemical and Ultrastructural Characteristics of an Interstitial Cell Type Different From ICC and Resident in the Muscle Coat of Human Gut." *Journal of Cellular and Molecular Medicine* 12, no. 5B: 1944–1955. https://doi.org/10.1111/j.1582-4934.2008.00461.x.

Pusztaszeri, M. P., W. Seelentag, and F. T. Bosman. 2006. "Immunohistochemical Expression of Endothelial Markers CD31, CD34, von Willebrand Factor, and Fli-1 in Normal Human Tissues." *Journal of Histochemistry and Cytochemistry* 54, no. 4: 385–395. https://doi.org/10.1369/jhc.4A6514.2005.

Ratajczak, M. Z., D. Ratajczak, and D. Pedziwiatr. 2016. "Extracellular Microvesicles (ExMVs) in Cell to Cell Communication: A Role of Telocytes." *Advances in Experimental Medicine and Biology* 913: 41–49. https://doi.org/10.1007/978-981-10-1061-3_3.

Rosa, I., P. Nardini, B. S. Fioretto, et al. 2023. "Immunohistochemical and Ultrastructural Identification of Telocytes in the Lamina Propria of Human Vaginal Mucosa." *Acta Histochemica* 125, no. 7: 152094. https://doi.org/10.1016/j.acthis.2023.152094.

Rosa, I., E. Romano, B. S. Fioretto, et al. 2021. "Scleroderma-Like Impairment in the Network of Telocytes/CD34(+) Stromal Cells in the Experimental Mouse Model of Bleomycin-Induced Dermal Fibrosis." *International Journal of Molecular Sciences* 22, no. 22: 12407. https://doi.org/10.3390/ijms222212407.

Rusu, M. C., D. Cretoiu, A. D. Vrapciu, et al. 2016. "Telocytes of the Human Adult Trigeminal Ganglion." *Cell Biology and Toxicology* 32, no. 3: 199–207. https://doi.org/10.1007/s10565-016-9328-y.

Rusu, M. C., V. S. Manoiu, D. Cretoiu, S. M. Cretoiu, and A. D. Vrapciu. 2018. "Stromal Cells/Telocytes and Endothelial Progenitors in the Perivascular Niches of the Trigeminal Ganglion." *Annals of Anatomy* 218: 141–155. https://doi.org/10.1016/j.aanat.2017.12.016.

Saharinen, P., and K. Alitalo. 2011. "The Yin, the Yang, and the Angiopoietin-1." *Journal of Clinical Investigation* 121, no. 6: 2157–2159. https://doi.org/10.1172/JCI58196.

Sanches, B. D. A., G. H. Tamarindo, J. D. S. Maldarine, et al. 2021. "Telocytes of the Male Urogenital System: Interrelationships, Possible Functions, and Pathological Implications." *Cell Biology International* 45, no. 8: 1613–1623. https://doi.org/10.1002/cbin.11612.

Sanches, B. D. A., G. H. Tamarindo, A. D. da Silva, et al. 2023. "Stromal Cell-Derived Factor 1 (SDF-1) Increases the Number of Telocytes in Ex Vivo and In Vitro Assays." *Histochemistry and Cell Biology* 160, no. 5: 419–433. https://doi.org/10.1007/s00418-023-02223-3.

Scaravilli, F., B. Giometto, L. Chimelli, and E. Sinclair. 1991. "Macrophages in Human Sensory Ganglia: An Immunohistochemical and Ultrastructural Study." *Journal of Neurocytology* 20, no. 7: 609–624. https://doi.org/10.1007/BF01215268.

Shoshkes-Carmel, M. 2024. "Telocytes in the Luminal GI Tract." *Cellular and Molecular Gastroenterology and Hepatology* 17, no. 5: 697–701. https://doi.org/10.1016/j.jcmgh.2024.02.002.

Smith, M., S. Chhabra, R. Shukla, et al. 2023. "The Transition Zone in Hirschsprung's Bowel Contains Abnormal Hybrid Ganglia With Characteristics of Extrinsic Nerves." *Journal of Cellular and Molecular Medicine* 27, no. 2: 287–298. https://doi.org/10.1111/jcmm.17659.

Stuart, T., A. Butler, P. Hoffman, et al. 2019. "Comprehensive Integration of Single-Cell Data." *Cell* 177, no. 7: 1888–1902e1821. https://doi.org/10.1016/j.cell.2019.05.031.

Tavares-Ferreira, D., S. Shiers, P. R. Ray, et al. 2022. "Spatial Transcriptomics of Dorsal Root Ganglia Identifies Molecular Signatures of Human Nociceptors." *Science Translational Medicine* 14, no. 632: eabj8186. https://doi.org/10.1126/scitranslmed.abj8186.

Thomas, P. D., D. Ebert, A. Muruganujan, T. Mushayahama, L. P. Albou, and H. Mi. 2022. "PANTHER: Making Genome-Scale Phylogenetics Accessible to All." *Protein Science* 31, no. 1: 8–22. https://doi.org/10.1002/pro.4218.

Traini, C., M. S. Fausssone-Pellegrini, D. Guasti, et al. 2018. "Adaptive Changes of Telocytes in the Urinary Bladder of Patients Affected by Neurogenic Detrusor Overactivity." *Journal of Cellular and Molecular Medicine* 22, no. 1: 195–206. https://doi.org/10.1111/jcmm.13308.

Valtcheva, M. V., B. A. Copits, S. Davidson, et al. 2016. "Surgical Extraction of human Dorsal Root Ganglia From Organ Donors and Preparation of Primary Sensory Neuron Cultures." *Nature Protocols* 11, no. 10: 1877–1888. https://doi.org/10.1038/nprot.2016.111.

Vandecasteele, T., P. Cornillie, K. Vandevelde, et al. 2017. "Presence of Ganglia and Telocytes in Proximity to Myocardial Sleeve Tissue in the Porcine Pulmonary Veins Wall." *Anatomia, Histologia, Embryologia* 46, no. 4: 325–333. https://doi.org/10.1111/ahe.12273.

Vannucchi, M. G., C. Traini, D. Guasti, G. Del Popolo, and M. S. Faussone-Pellegrini. 2014. "Telocytes Subtypes in Human Urinary Bladder." *Journal of Cellular and Molecular Medicine* 18, no. 10: 2000–2008. https://doi.org/10.1111/jcmm.12375.

Wang, J., M. Jin, W. H. Ma, Z. Zhu, and X. Wang. 2016. "The History of Telocyte Discovery and Understanding." *Advances in Experimental Medicine and Biology* 913: 1–21. https://doi.org/10.1007/978-981-10-1061-3_1.

Wangzhou, A., C. Paige, S. V. Neerukonda, et al. 2021. "A Ligand-Receptor Interactome Platform for Discovery of Pain Mechanisms and Therapeutic Targets." *Science Signaling* 14, no. 674: eabe1648. https://doi.org/10.1126/scisignal.abe1648.

Wishahi, M., E. Hafiz, A. M. K. Wishahy, and M. Badawy. 2021. "Telocytes, c-Kit Positive Cells, Smooth Muscles, and Collagen in the Ureter of Pediatric Patients With Congenital Primary Obstructive Megaureter: Elucidation of Etiopathology." *Ultrastructural Pathology* 45, no. 3: 257–265. https://doi.org/10.1080/01913123.2021.1954734.

Xiao, J., and Y. Bei. 2016. "Decoding Telocytes." *Advances in Experimental Medicine and Biology* 913: 23–39. https://doi.org/10.1007/978-981-10-1061-3_2.

Xu, Y., H. Tian, G. Qiao, and W. Zheng. 2021. "Telocytes in the Atherosclerotic Carotid Artery: Immunofluorescence and TEM Evidence." *Acta Histochemica* 123, no. 2: 151681. https://doi.org/10.1016/j.acthis.2021.151681.

Yrigoin, K., K. N. Bernard, M. A. Castano, O. Cleaver, S. Sumanas, and G. E. Davis. 2024. "Enhancing Human Capillary Tube Network Assembly and Maturation Through Upregulated Expression of Pericyte-Derived TIMP-3." *Frontiers in Cell and Developmental Biology* 12: 1465806. https://doi.org/10.3389/fcell.2024.1465806.

Zhang, Z., and L. Y. Li. 2012. "TNFSF15 Modulates Neovascularization and Inflammation." *Cancer Microenvironment* 5, no. 3: 237–247. https://doi.org/10.1007/s12307-012-0117-8.

Zhou, S., S. Zhang, Y. Wang, et al. 2015. "MiR-21 and miR-222 Inhibit Apoptosis of Adult Dorsal Root Ganglion Neurons by Repressing TIMP3 Following Sciatic Nerve Injury." *Neuroscience Letters* 586: 43–49. https://doi.org/10.1016/j.neulet.2014.12.006.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.