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RESEARCH ARTICLE

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Telocytes are associated with tissue remodeling and angiogenesis during the postlactational involution of the mammary gland in gerbils

Bruno D. A. Sanches[1] | Ellen C. R. Leonel[2] | Juliana S. Maldarine[1] | Guilherme H. Tamarindo[1] | Caroline N. Barquilha[3] | Sérgio L. Felisbino[3] | Rejane M. Goés[2] | Patricia S. L. Vilamaior[2] | Sebastião R. Taboga[2]

[1]Department of Structural and Functional Biology, State University of Campinas, Campinas, São Paulo, Brazil

[2]Laboratory of Microscopy and Microanalysis, Department of Biology, University of Estadual Paulista (UNESP), São José do Rio Preto, São Paulo, Brazil

[3]Department of Structural and Functional Biology, Institute of Biosciences, University of Estadual Paulista (UNESP), Botucatu, São Paulo, Brazil

Correspondence
Sebastião R. Taboga, Laboratory of Microscopy and Microanalysis, Department of Biology, São Paulo State University, 2265, Cristóvão Colombo St., Jardim Nazareth, São José do Rio Preto, São Paulo 15054-000, Brazil.
Email: sebastiao.taboga@unesp.br

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Abstract

The postlactational involution of the mammary gland is a complex process. It involves the collapse of the alveoli and the remodeling of the extracellular matrix, which in turn implies a complex set of interrelations between the epithelial, stromal, and extracellular matrix elements. The telocytes, a new type of CD34-positive stromal cell that differs from fibroblasts in morphological terms and gene expression, were detected in the stroma of several tissues, including the mammary gland; however, their function remains elusive. The present study employed three-dimensional reconstructions and immunohistochemical, ultrastructural, and immunofluorescence techniques in histological sections of the mammary gland of the Mongolian gerbil during lactation and postlactational involution to evaluate the presence of telocytes and to investigate a possible function for these cells. By means of immunofluorescence assays for CD34 and c-kit, major markers of telocytes, and also through morphological and ultrastructural evidences, telocytes were observed to surround the mammary ducts and collapsing alveoli. It was also found that these cells are associated with matrix metalloproteinase 9, which indicates that telocytes can play a role in extracellular matrix digestion, as well as vascular endothelial growth factor, a factor that promotes angiogenesis. Together, these data indicate that telocytes are a distinct cell type in the mammary gland and, for the first time, show that these cells possibly play a role in tissue remodeling and angiogenesis during the postlactional involution of the mammary gland.

KEYWORDS

CD34, extracellular matrix remodeling, gerbil, mammary gland involution, telocytes, TGF-β1, VEGF

1 | INTRODUCTION

The mammary gland is a highly regenerative gland and its presence is one of the characteristics that distinguish mammals from other animals. It produces milk, which is a complex apocrine secretion,

Abbreviations: 3D, three-dimensional; CD31 and 34, Cluster Differentiation 31 and 34; HE, hematoxylin-eosin; MMP9, matrix metalloproteinasenonbreakingspace9; PCNA, proliferating cell nuclear antigen; TEBs, terminal end buds; TEM, transmission electron microscopy; TGFβ1, transforming growth factor-β1; TNFR1, tumor necrosis factor receptor 1; VEGF, vascular endothelial growth factor; α-SMA, α-smooth muscle actin.

that provides nutrition for puppies, as well as contribute to their immune defense (Goldman, 2012). Unlike other glands, which function continuously, such as the prostate, salivary, and lacrimal glands, the mammary gland undergoes expansion cycles, in which its secretory units, mammary alveoli, are formed in a process called alveologenesis, and they regress in a process called involution (Paine & Lewis, 2017). The dynamics of the expansion/involution of the gland have developed over evolutionary time in a finely coordinated way with pregnancy and the beginning of the postnatal life of the puppies. Although in some species there is breastfeeding by the males (Kunz & Hosken, 2009), in most species, the male mammary gland remains rudimentary, with a duct network without alveoli. In adolescence, this rudimentary duct network, common to male and female mammals, develops only in females, when terminal end buds (TEBs) are formed. TEBs are the ends of the ducts that will branch off to form a more complex network of ramifications that end in blind ducts in rodents and acini in humans. This process occurs under the influence of estradiol and insulin growth factor 1 (IGF-1); however, it is only under the influence of progesterone and prolactin in late pregnancy that the alveoli of the mammary gland will be formed and will produce milk (Macias & Hinck, 2012).

Lactation time varies between species; for example, it lasts on average about 6 months in humans, whereas in mice, it only lasts about 21 days (Sengupta, 2013) and ends up in the weaning. In evolutionary terms, the weaning phase arrives when the costs of maintaining the current offspring exceed those of the acquisition of new offspring, which results in the so-called weaning conflict, in which the puppy tries to continue breastfeeding, while the lactating female becomes less receptive (Enríquez, Hötzel, & Ungerfeld, 2011; Packard, Mech, & Ream, 1992). In this way, the maintenance of lactation evolved in a regulated way by environmental signs; with the absence of the mechanical stimulus of suction of the nipples at weaning, the gland enters a reversible phase of involution and, as well studied in rats, after 48 h without the stimulus of suckling, follows an irreversible phase of gland involution in which the alveoli collapse and the gland regress to its prepregnancy form. In the reversible phase, there is an increase in apoptotic activity in the epithelium, which is mediated by proteins of the signal transducer and activator of transcription family and the progressive detachment of alveolar cells and the accumulation of shed cells in the lumens. In the irreversible phase, the stroma plays a decisive role in the activation of matrix metalloproteinases (MMPs), along with the progressive digestion of the extracellular matrix of the mammary gland stroma (Macias & Hinck, 2012).

Studies have shown great complexity in stromal cells, evidenced by the heterogeneity of fibroblasts in terms of morphological and possibly functional markers, especially on the skin (Driskell & Watt, 2015; Lemons et al., 2010; Lynch & Watt, 2018). As well as the presence of several types of CD34-positive cells, such as vascular endothelial progenitors (Özen, Boix, & Paul, 2012), lymphatic endothelial progenitors (Rossi et al., 2010; Tan et al., 2014), and fibroblast-like cells, the so-called telocytes, were found in various organs. Telocytes were initially described by Popescu and Faussone-Pellegrini (2010); these cells have a characteristic morphology, with thin cytoplasmic extensions, the telopodes that have dilations called podoms, which in turn have organelles such as mitochondria and endoplasmic reticules, something not found in fibroblasts. In addition, telocytes differ from fibroblasts, pericytes (Bei et al., 2015), and mesenchymal stem cells (Zheng et al., 2013) in terms of proteome and gene expression (Kang et al., 2015; Zheng et al., 2014).

These cells have been described in several organs, such as the kidneys (Qi et al., 2012), the skeletal muscle (Marini, Manetti, Rosa, Ibba-Manneschi, & Sgambati, 2018), the skin (Ceafalan, Gherghiceanu, Popescu, & Simionescu, 2012), the joints (Rosa, Marini, Guasti, Ibba-Manneschi, & Manetti, 2018), the prostate (Corradi et al., 2013), the jejunum (Zani et al., 2018), the sclera (Petrea, Crăiţoiu, Vrapciu, Mănoiu, & Rusu, 2018), the heart (Kostin, 2010), the ureter (Dobra, Vrapciu, Pop, Petre, & Rusu, 2018), and the mammary gland. The functions proposed for telocytes vary from organ to organ. In the mammary gland, functions have been suggested in paracrine signaling, in the maintenance of stem cell niches, and in immune response (Hostiuc, Negoi, & Perlea, 2018); however, the exact role of telocytes in the mammary gland remains unclear. Among laboratory rodents, the Mongolian gerbil is known for its easy handling and high susceptibility to pathological conditions (Sanches et al., 2014, 2020; Santos & Taboga, 2006), being used in experiments on physiology, development, and pathogenesis in several organs (Biancardi, Dos Santos, de Carvalho, Sanches, & Taboga, 2017; Christante, Taboga, Pinto-Fochi, & Góes, 2013; Perez et al., 2012; Sanches et al., 2017; Santos et al., 2008), including the mammary gland. It was found that the adult mammary gland in gerbils presents a more proliferative profile during reproductive life compared to other laboratory rodents, such as rats and mice, in addition to a possible greater susceptibility to tumorigenesis (Leonel, Falleiros, Campos & Taboga, 2017), but the dynamics of mammary gland involution in the gerbil and the possible function of telocytes remain poorly understood. Therefore, this study aims to evaluate the presence of telocytes in the mammary gland in gerbils during postlactational involution, as well as a possible role for these cells.

2 | MATERIAL AND METHODS

2.1 | Animals and experimental design

The animals were provided by São Paulo State University (UNESP, São José do Rio Preto). Gerbils were housed in a temperature-controlled (25°C) room on a 12 h light/dark cycle. All the animals were housed in polyethylene cages, with ad libitum access to filtered water and rodent food. Animal handling and experiments were performed according to São Paulo State University ethical guidelines of the (UNESP, Ethical Committee number 115/2015 CEUA), following the Guide for the Care and Use of Laboratory Animals. We used six adult females (between 5 and 6 months old) Mongolian gerbils (*Meriones unguiculatus*, Muridae: Gerbillinae) 7 days after weaning on the 28th day after the birth of the pups (Norris & Adams, 1972) and a

group of six lactating females at the 14th day of lactation. The mammary glands of postlactational females were used for morphological analysis and three-dimensional (3D) reconstructions, as well as immunohistochemistry and immunofluorescence assays, to describe the mammary gland involution period in the gerbil, as well as to evaluate the presence of telocytes and a possible function for these cells. The mammary glands of the lactating females were employed for 3D reconstruction for comparative purposes, immunofluorescence assays, and ultrastructural analyses. The animals were killed by a lethal injection containing a mixture of an anesthetic, ketamine (100 mg/kg bw, Dopalen, Vetbrands, Brazil), and a muscle relaxant, xylazine (11 mg/kg bw, Rompun, Bayer, Brazil).

2.2 | Histological processing

After dissection, the mammary glands were collected and fixed in 4% buffered paraformaldehyde or Karnovsky (5% paraformaldehyde solution and 2.5% glutaraldehyde in Sörensen pH 7.2 0.1 M phosphate buffer), washed in water, dehydrated in ethanol, clarified in xylol, and then embedded in Paraplast (Histosec; Merk). The organs were sectioned at 3–5 μm and histological slides were mounted; some of them were stained by the hematoxylin-eosin (HE) histochemical technique for general morphological studies, as well as by Picrosirius Red for collagen fiber detection (Junqueira, Bignolas, & Brentani, 1979).

2.3 | 3D reconstructions

3D reconstructions were performed to obtain a 3D detailing of the mammary gland involution in the gerbil, as well as of the mammary gland on the 14th day of lactation. Histological sections of the mammary glands were cut serially, 5-μm thick, and were then mounted in histological slides and stained with H&E. These were then analyzed and photographed through an Olympus BX60 light microscope (Olympus) coupled to a computer with the DP-BSW V3.1 software (Olympus) AMS and Image Pro 6.1 software (Media Cybernetics) for Windows (Media Cybernetics). Later, the images were reconstructed through Reconstruct software (Fiala, 2005).

2.4 | Immunofluorescence assays

Immunofluorescence of paraffin-embedded tissue sections was performed using the protocol described in Lima et al. (2015). The mammary glands of the postlactational females were fixed by immersion in 4% paraformaldehyde (buffered in 0.1 M phosphate, pH 7.4) for 24 h. After fixation, the tissues were washed in water, dehydrated in ethanol series, paraffin-embedded (Histosec; Merck), and sectioned at 5 μm on a Leica microtome (Leica RM2155). These sections of gerbil mammary glands were subjected to immunofluorescence assays to evaluate the presence of telocytes and a possible function for these cells. Immunofluorescence assays for CD34 (mouse polyclonal, immunoglobulin G [IgG], B-6, sc74499; Santa Cruz Biotechnology), c-kit (rabbit polyclonal, IgG, C-19, sc168; Santa Cruz Biotechnology), CD31 (rabbit polyclonal, IgG, M-20, sc-1506; Santa Cruz Biotechnology), α-smooth muscle actin (α-SMA; rabbit polyclonal, IgG, I-19, sc-1616; Santa Cruz Biotechnology), transforming growth factor-β1 (TGF-β1; rabbit polyclonal, IgG, V, sc146; Santa Cruz Biotechnology), MMP9 (mouse monoclonal, IgG, MMP9 2C3, sc-21733), and vascular endothelial growth factor (VEGF; rabbit polyclonal, IgG, A-20, sc-152; Santa Cruz Biotechnology) were therefore performed; these antibodies were incubated at a dilution of 1:100 overnight. On the following morning, the sections were incubated with goat anti-mouse antibody (FITC, sc-2011; Santa Cruz Biotechnology) and goat anti-rabbit (sc-2780, Texas Red; Santa Cruz Biotechnology) and secondary antibodies diluted 1:200 in 1% bovine serum albumin for 2 h at room temperature and then stained with 4′,6-diamidino-2-phenylindole (F36924; Life Technology). Histological sections were analyzed with a ZeissImager M2 fluorescence microscope (Zeiss) coupled with AxioVision software (Zeiss).

2.5 | Immunohistochemistry assays

Sections of the mammary glands in postlactational involution were submitted to immunohistochemistry for proliferating cell nuclear antigen (PCNA) detection using the protocol adopted in Fochi, Santos, Goes, and Taboga (2013). Antibodies (at a dilution of 1:100) were used for the detection of PCNA (monoclonal mouse IgG2a, PC10, sc-56; Santa Cruz Biotechnology). RE7200-K (Leica Biosystems) was used as the secondary antibody. The incubation time was 45 min at 37°C. Later, the assays were performed using diaminobenzidine (DAB) and the sections were counterstained with Harris hematoxylin. Histological sections were analyzed with the Olympus BX60 (Olympus) light microscope. Negative controls were obtained by omission of the step of incubating the primary antibody.

2.6 | Quantification of immunohistochemistry assays

For the purpose of quantification, immunohistochemical assays for PCNA were performed to evaluate the proliferative profile of the mammary gland during postlactational involution; the total number of cells in 30 fields, derived from three different animals, was counted randomly (at ×20 magnification); this was done according to the methodology described in Fochi et al. (2013). The cells were separated into positively and negatively labeled; a minimum of 1000 cells was counted per group, and then the percentage of immunostaining was calculated as the number of positive cells divided by the total number of cells. Quantitative results are expressed in a graph.

2.7 | Ultrastructural analysis

Ultrastructural analysis was performed using the protocol described by Corradi et al. (2013). Fragments of the mammary glands at two different stages, the 14th day of lactation and the 7th day after weaning, were minced into small pieces and fixed by immersion in 3% glutaraldehyde plus 0.25% tannic acid solution in Millonig's buffer, pH 7.3, containing 0.54% glucose for 24 h. After washing with the same buffer, the samples were post-fixed with 1% osmium tetroxide for 1 h, washed in buffer, dehydrated in a graded acetone series, and embedded in Araldite resin. Ultrathin sections (50–75 nm) were prepared using a diamond knife and stained with 2% alcoholic uranyl acetate for 30 min, followed by 2% citrate in a 1 M sodium hydroxide solution for 10 min. Samples were evaluated by electron microscopy using a LEO—Zeiss 906 TEM at 80 kV.

2.8 | Statistical analysis

The data were initially studied by Student's *t* test, with a significance level of 5% ($p \leq .05$). The statistical tests were performed with Statistica 7.0 software (StarSoft Inc.). This test was used to verify differences in cell proliferation between the stromal and epithelial compartments of the mammary gland.

3 | RESULTS

3.1 | CD34 and c-kit immunofluorescence assays

The involution of the mammary gland of Mongolian gerbils occurs similarly to that seen in other species of laboratory rodents, with the progressive collapse of the mammary gland alveoli after weaning (Figure 1b) in comparison to the lactational phase, in which the alveoli are producing milk and have large volumes (Figure 1a). CD34-positive cells showed colocalization with c-kit in blood vessels, and some exclusively CD34-positive cells were observed at the periphery of the mammary gland alveoli on the 14th day of lactation (Figure 2a–d). One week after weaning, the colocalization of CD34 and c-kit was also seen in blood vessels, which are quite evident and may indicate an increase in the vascularization of the gland during involution. Immunolabeling for CD34 and c-kit was also seen in the perialveolar region with fibrillar-like dispositions, typical of the so-called telocytes (Figure 2e–h).

3.2 | TGF-β1, CD34, and MMP9 immunofluorescence assays

Immunostaining for α-SMA reveals the layer of myoepithelial cells that surround the luminal cells of the mammary gland alveoli; TGF-β1 immunolabeling was seen around these cells (Figure 3a–d); in that same region, exclusively CD34 positive cells, which do not present colocalization with CD31, were observed; they were therefore not blood vessels, and may possibly be telocytes (Figure 3e–h). In addition, in this region, there was immunostaining for MMP9, which may indicate that these cells act in the stromal remodeling typical of gland involution (Figure 3i–k).

3.3 | Histochemistry

During the involution of the mammary glands, possible telocytes were observed in the interstice between histological sections

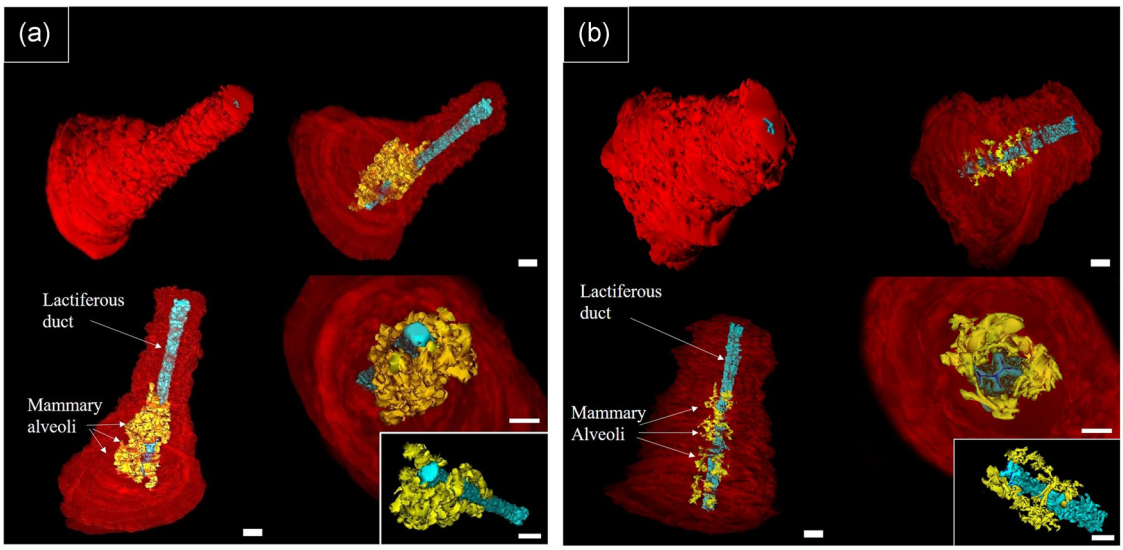

FIGURE 1 Three-dimensional reconstructions of serial histological sections of the Mongolian gerbil mammary gland on the 14th day of lactation and the 7th day after weaning. (a) Mammary gland on the 14th day of lactation, in which the presence of developed alveoli around the lactiferous duct is observed. (b) In the mammary gland seven days after weaning, the presence of collapsing alveoli around the lactating duct is verified. Color notations: red (nipple), yellow (mammary gland alveoli), cyan (lactiferous duct), white bar (200 μm)

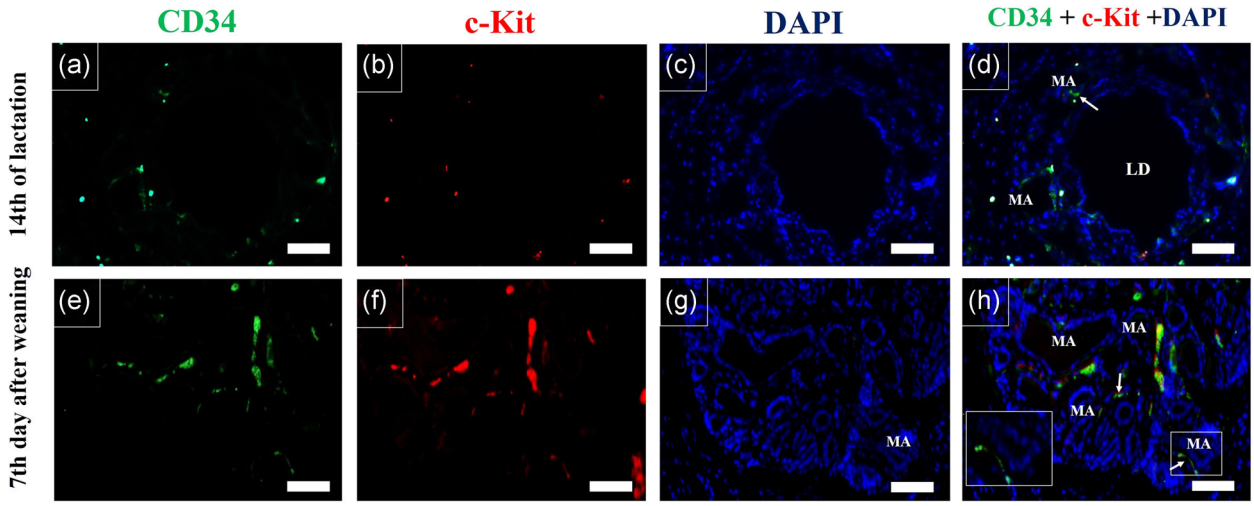

FIGURE 2 Double immunofluorescence assays for CD34 and c-kit in histological sections of the Mongolian gerbil mammary gland during lactation and postlactational involution. (a–d) On the 14th day of lactation, sparse immunolabeling for CD34 is seen around the mammary gland alveoli. (e–h) On the 7th day after weaning, there is colocalization between the CD34 and the c-kit in fibrillar-like cells around the collapsing alveoli. Arrows, CD34 positive cells in the perialveolar region; DAPI, 4′,6-diamidino-2-phenylindole; LD, lactiferous ducts; MA, mammary gland alveoli. Bars = 50 μm

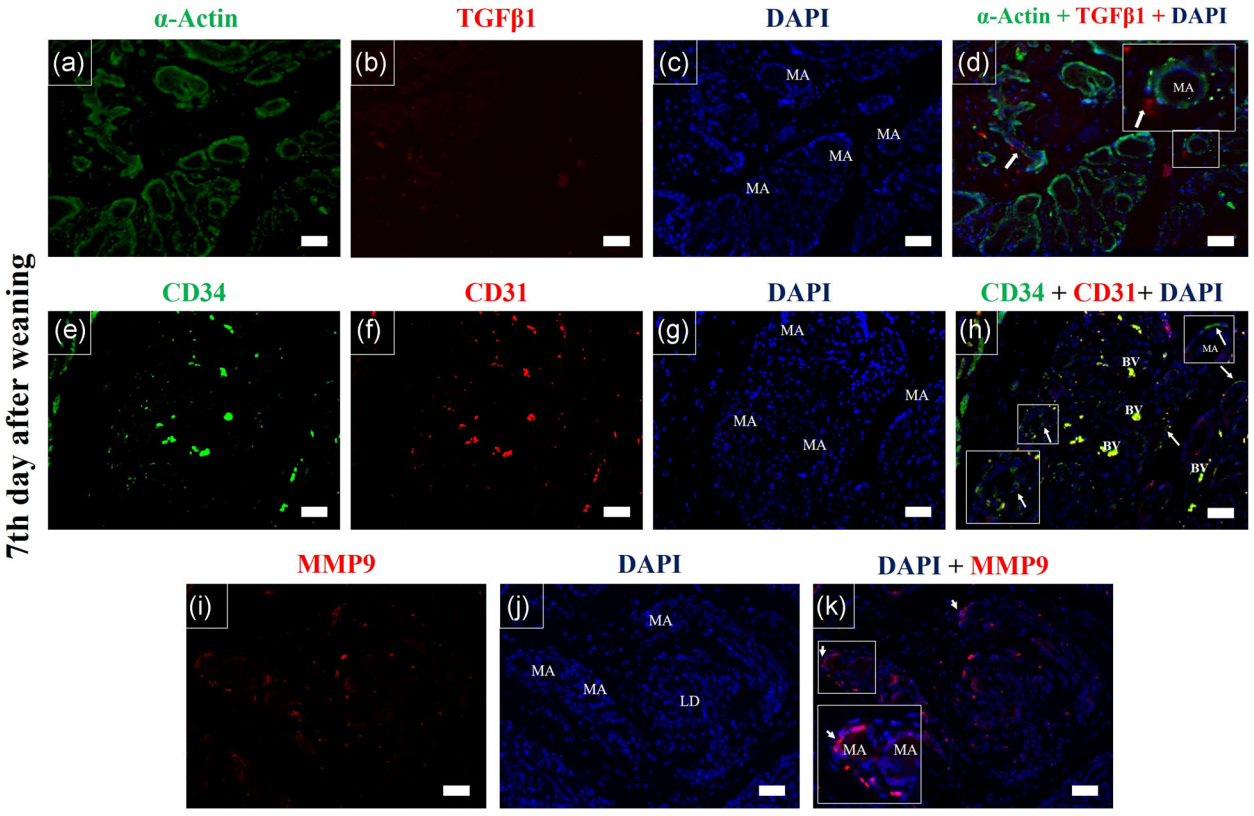

FIGURE 3 Double immunofluorescence assays for α-SMA and TGF-β1, for CD34 and c-kit and for MMP9 in histological sections of the Mongolian gerbil mammary gland during postlactational involution. (a–d) α-SMA immunolabeling was observed in myoepithelial cells in mammary gland alveoli. TGF-β1 immunolabeling is seen dispersed in blood vessels and at the periphery of collapsing alveoli in addition to the lactiferous duct. (e–h) CD34 and CD31 colocalization was observed in blood vessels, and there are few exclusively CD34-positive cells at the periphery of collapsing alveoli. (i–k) MMP9 immunolabeling is verified in the vicinity of collapsing alveoli. Arrow, exclusive CD34 immunolabeling at the periphery of collapsing alveoli; arrowhead, MMP9 immunolabeling at the periphery of collapsing alveoli; BV, blood vessels; large arrow, TGF-β1 immunolabeling; LD, lactiferous duct; MA, mammary gland alveoli; MMP9, matrix metalloproteinase 9; TGF-β1, transforming growth factor-β1; α-SMA, α-smooth muscle actin. Bars = 50 μm

stained with H&E (Figure 4a–c). Collagen fibers were deposited around the lactiferous duct (Figure 4d,e), cell death and shed cells were verified in the collapsing alveoli (Figure 4f). Digested collagen fibers were observed in the periphery of the prostatic alveoli, which indicates the remodeling of the extracellular matrix, and possible telopodes were observed in this region (Figure 4g–i).

3.4 | Immunohistochemistry

One week after weaning, the stroma and epithelium of the gerbil mammary gland remain proliferative, although the proliferation was greater in the stroma than in the epithelium, which coincides with the tissue remodeling seen in the stroma; the myoepithelial cells remain proliferative, while the loss of the secretory epithelium occurs (Figure 5a–f).

3.5 | TNFR1 and VEGF immunofluorescence assays

The immunolabeling for TNFR1 during gland involution, a factor implicated in inflammation and apoptosis, was restricted to blood vessels (Figure 6a–h). The stimulus for angiogenesis indicated by VEGF immunolabeling was limited to blood vessels during lactation (Figure 6i–l), but it was also verified in the perialveolar region during gland involution (Figure 6m–p), the same region in which telocytes were found, which may indicate a role for these cells in angiogenesis during gland involution.

3.6 | Ultrastructural data

The ultrastructural data indicated the presence of telocytes in the mammary gland, both in the perialveolar and in the interstitial

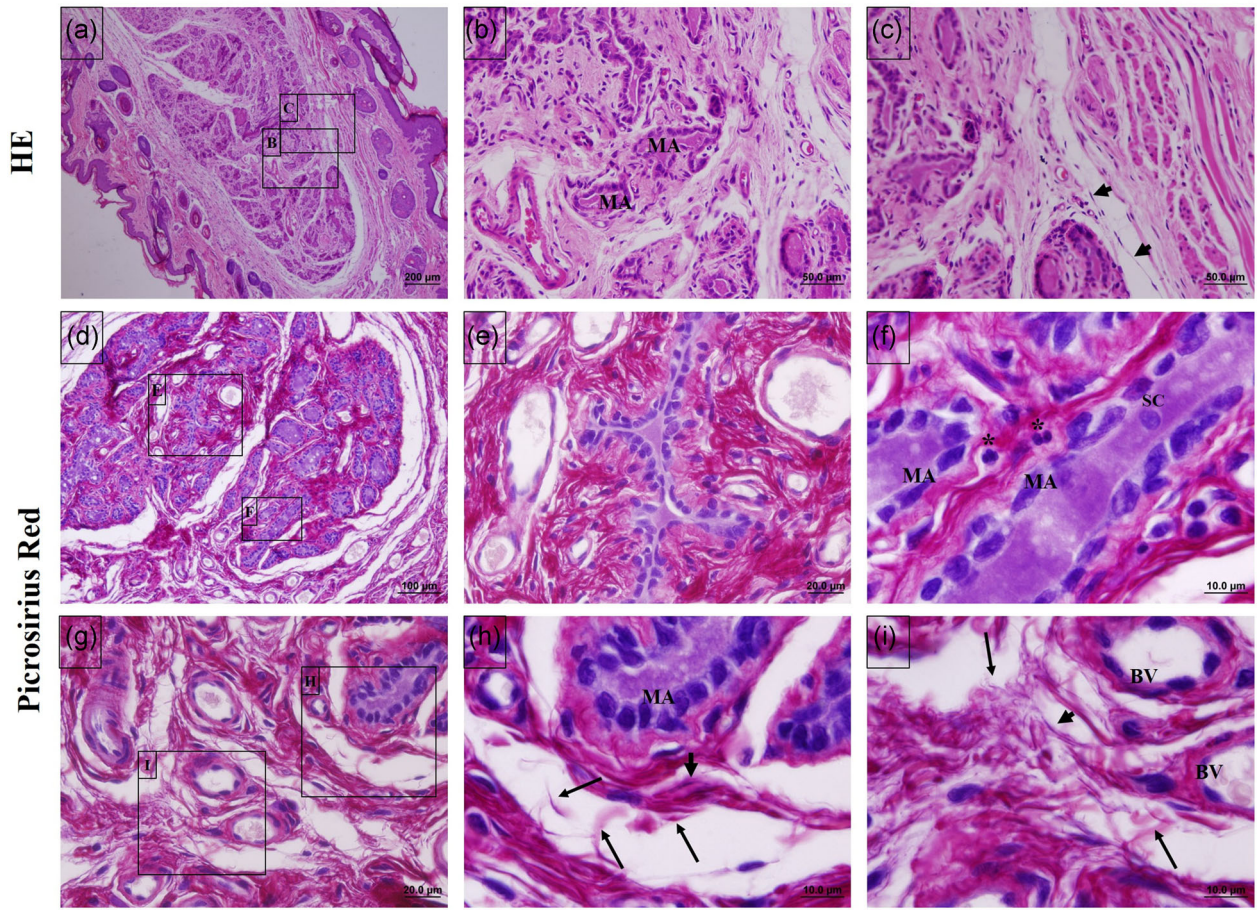

FIGURE 4 Histological sections of the mammary gland in postlactational involution of the Mongolian gerbil on the 7th day after weaning. (a) Histological section of the involutional mammary gland stained with H&E. (b) Detail of a collapsing alveolus. (c) Possible telocytes in the mammary gland interstitium. (d) Involutional mammary gland stained with Picrosirius Red for the detection of collagen fibers. (e) Deposition of collagen fibers around the lactiferous duct. (f) Collapsing alveoli in which cell death and shed cells are observed. (g) Detail of intense remodeling of the extracellular matrix. (h) Detail of digested collagen fibers at the periphery of a collapsing alveolus. A possible telocyte interspersed in the collagen fibers is verified. (i) Details of blood vessels in the mammary gland and of extracellular matrix remodeling; a possible telopode is observed. Arrows, digested collagen fibers; arrowhead, possible telocytes; asterisks, cell death; H&E, hematoxylin and eosin; MA, collapsing mammary alveoli

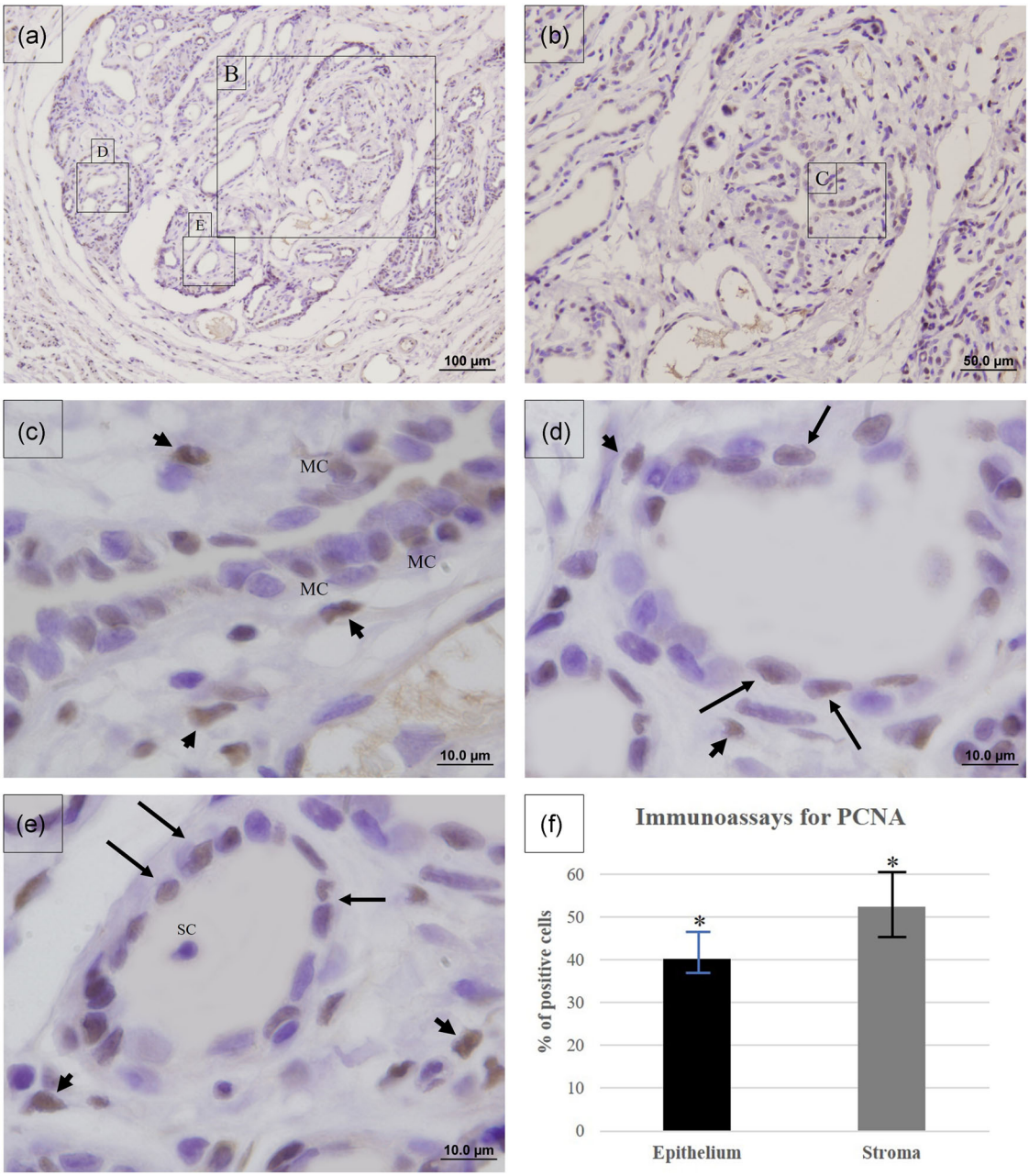

FIGURE 5 Immunohistochemical assays for PCNA in histological sections of the mammary gland on the 7th day after weaning during postlactational involution. (a) PCNA immunostaining in the mammary gland. (b) PCNA immunostaining in both the epithelium and the stroma of the mammary gland. (c–e) PCNA immunostaining in myoepithelial cells and stromal cells. (f) Both the epithelium and the stroma have a high proliferative profile, although it is higher in the stroma. Arrow, epithelial immunostaining; arrowhead, stromal immunostaining; asterisk, significant difference; MC, myoepithelial cells; PCNA, proliferating cell nuclear antigen; SC, shed cells

regions, during lactation; these cells diverge from other stromal cells such as fibroblasts because they have long cytoplasmic projections, the telopodes, which are divided between dilations, podoms, which in turn have organelles such as mitochondria, alternated by fibrillar sections, the podomeres. In addition, these cells were seen in close contact with myoepithelial cells (Figure 7a–d). This ultrastructural evidence, together with the immunofluorescence and histological data, corroborated the idea that CD34 positive cells in the peri-alveolar region of the mammary gland are effectively telocytes.

4 | DISCUSSION

The presence of CD34, which is a transmembrane glycoprotein on the surface of immature hematopoietic cells, has been known for a long time (Wood, May, Healy, Enver, & Morriss-Kay, 1997). But, in addition to hematopoietic cells, there is evidence that CD34 is present on the surface of a series of adult progenitor cells in several organs (Sidney, Branch, Dunphy, Dua, & Hopkinson, 2014). There are CD34-positive cells of progenitor potential related to blood vessels,

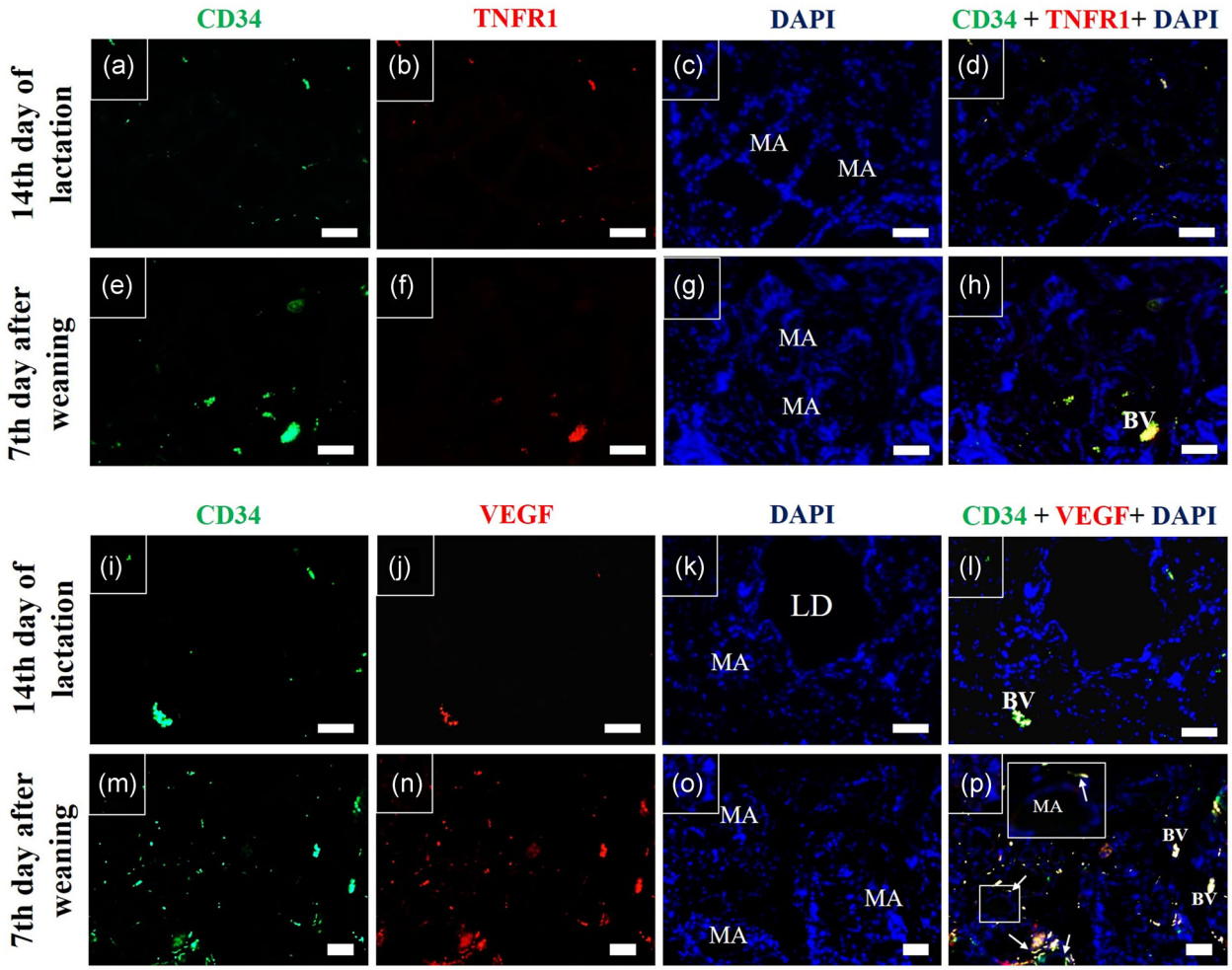

FIGURE 6 Double immunofluorescence assays for CD34 and TNFR1 and for CD34 and VEGF in histological sections of the Mongolian gerbil mammary gland during postlactational involution. (a–d) On the 14th day of lactation, TNFR1 immunolabeling colocalizes with that of CD34 and it is observed in blood vessels; this pattern is also seen on the 7th day after weaning (e–h). (i–l) The CD34 immunolabeling colocalizes with VEGF in blood vessels on the 14th day of lactation. (m–p) On the 7th day after weaning, the colocalization of CD34 and VEGF is seen in blood vessels, but also in the perialveolar region. Arrow, colocation of CD34 with VEGF in the perialveolar region; BV, blood vessel; MA, mammary alveoli; VEGF, vascular endothelial growth factor. Bar = 50 μm

such as perivascular mesenchymal stem cells (Özen et al., 2012), in addition to lymphatic endothelial progenitors (Rossi et al., 2010; Tan et al., 2014) and, finally, CD34-positive stromal cells, which comprise so-called telocytes (Popescu & Faussone-Pellegrini, 2010). A recent study pointed out that part of what we call telocytes would be actually pre-lymphatic endothelial cells, which would be CD34- and CD31-positive cells (Toader et al., 2019). But a more in-depth study carried out later showed that these cells are close to but distinct from telocytes and that they are not CD34 positive (Rosa, Marini, Sgambati, Ibba-Manneschi, & Manetti, 2020). In terms of distinguishing telocytes from other CD34-positive cells, the use of the CD31 immunolabelling can be considered an important tool, since telocytes are negative for this marker (Felisbino et al., 2019; Suciu, Popescu, Kostin, & Popescu, 2012). Our data show that in the perialveolar region, fibrillar-like CD34 immunolabeling is found; ultrastructural data show the presence of cells with cytoplasmic projections, the telopodes, divided between dilated sections, the podoms, with organelles such as mitochondria, and fibrillar sections, the podomers. Together, these data indicate the presence of telocytes in the perialveolar region.

An increasing number of studies demonstrate the importance of telocytes in the stroma physiology in several organs (Ceafalan et al., 2012; Corradi et al., 2013; Marini et al., 2018; Rosa et al., 2018). There is evidence that these cells participate actively in stromal tissue organization, so that the telopode network would be related to the compartmentalization of stromal microenvironments, enabling the spatial restriction of differentiation and growth factors, which is essential for tissue organization during morphogenesis, such as in the heart (Bani, 2016) and in the prostate (Sanches et al., 2017). In the case of the prostate, telocytes also actively secrete TGF-β1; this factor

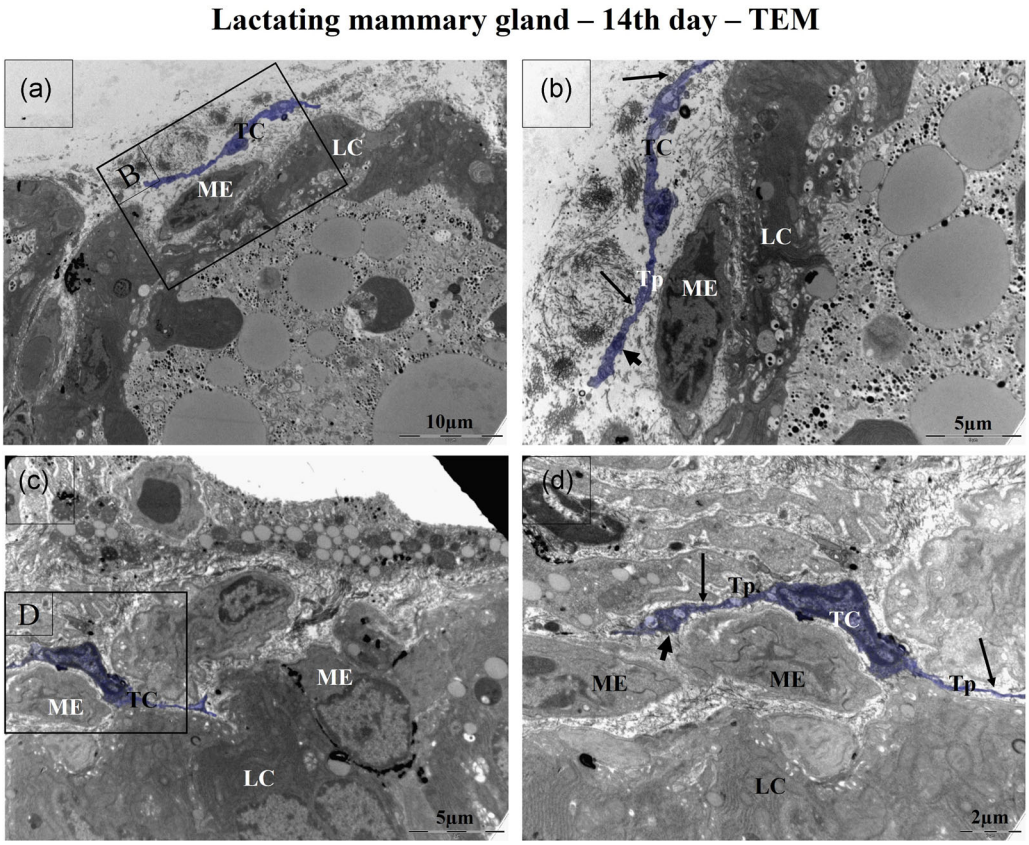

FIGURE 7 Ultrastructure of the mammary gland on the 14th day of lactation. (a,b) In the perialveolar region and close to the interstice, a telocyte is observed with its long cytoplasmic projection, the telopode, which is divided into dilations, the podoms, which in turn have organelles such as mitochondria, and fibrillar-like sections, the podomeres. (c,d) A telocyte is observed in the perialveolar region with a small telopode. Arrows, podomers—fibrillar-like sections of telopodes; arrowhead, podoms—dilated sections of telopodes; LC, luminal cells; ME, myoepithelial cells; TC, dark blue (artificially colored telocytes; Tp, Telopodes

has been associated with smooth muscle differentiation (Hong et al., 2004; Sanches, Corradi, Vilamaior, & Taboga, 2016). Our data indicate that TGF-β1 is also secreted by telocytes in the mammary gland, which could confer a role for these cells in the differentiation and/or maintenance of myoepithelial cells, as well as in the decrease of epithelial proliferation (Macias & Hinck, 2012); there is also evidence that TGF-β1 positively regulates the MMP9 (Sun et al., 2008), an enzyme that digests the extracellular matrix, participating in stromal tissue remodeling.

Telocytes can also play a role in remodeling and maintaining the extracellular matrix in the heart muscle, especially through digestion of the extracellular matrix in the myocardium (Kostin, 2010). It was verified by genomic analysis that telocytes express genes from several MMPs (Zheng et al., 2013); in addition, the reduction in the number of telocytes has been associated with fibrosis in the intestine (Manetti, Rosa, Messerini, & Ibba-Manneschi, 2015) and in the myocardium (Kostin, 2010), which corroborates the role of telocytes in digestion and remodeling of the extracellular matrix, as well as placing this cell type as a potential therapeutic target for the treatment of fibrosis. Our data on the postlactational involution of the mammary gland demonstrate the presence of MMP9 in the perialveolar region, which may corroborate the role of telocytes in the digestion of the extracellular matrix and in the remodeling of the stroma. In the mammary gland, the intense remodeling of the stroma of the mammary gland is fundamental for the reorganization of the mammary ducts that occurs concurrently with the collapse of the alveoli; this process allows the gland to return to a state similar to that seen in the period before pregnancy and makes it able to undergo a new alveologenesis phase along with the onset of a new pregnancy (Macias & Hinck, 2012; Martinson, Jindal, Durand-Rougely, Borges, & Schedin, 2015).

In addition, our data indicate that the mammary gland in the gerbil remains highly proliferative in postlactational involution, which corroborates a previous study (Leonel et al., 2017), conversely to what is seen in other laboratory rodents in which there is a decrease in epithelial proliferation during this period (Allan, Beattie, & Flint, 2004). Our data also indicate an intense process of angiogenesis in the gland during involution. The presence of VEGF in the perialveolar region was verified in colocalization with CD34, which indicates the role of telocytes in angiogenesis in the mammary gland during postlactational involution, which has also been proposed for telocytes in skeletal muscle (Suciu et al., 2012) and for those in the lungs (Hussein & Mokhtar, 2018). In addition, it has been hypothesized that telocytes would be able to differentiate into smooth muscle cells

(Bei et al., 2015) or even into fibroblasts or myofibroblasts (Díaz-Flores et al., 2016) and that they would belong to a wide subset of CD34-positive cells that have progenitor potential (Sidney et al., 2014), which may indicate that a portion of these cells could possibly act in tissue reorganization through differentiation into fibroblasts or smooth muscle cells; however, further studies are necessary to assess this potential.

It is also worth mentioning the parallels raised between mammary gland involution and tumorigenesis (Clarkson & Watson, 2003; Martinson et al., 2015), especially with regard to the transcription of common genes in both processes, as well as to the increase the probability of breast cancer after pregnancy (Schedin, 2006). Thus, the study of mammary gland involution becomes particularly interesting, and there is also the possibility that telocytes may perform a role in tumorigenesis. Recently, it was observed that telocytes promoted the proliferation of breast cancer-derived cells in vitro (Mou et al., 2012), as well as an increase in the presence of telocytes was verified in uterine leiomyomas (Othman et al., 2016). Telocytes can also be the cells of origin of certain types of cancer; for example, prostate tumors that have higher CD34 and c-kit immunolabeling correlate to a higher degree on the Gleason scale, making them more aggressive than those that have less immunolabeling for these factors (Foroozan, Roudi, Abolhasani, Gheytanchi, & Mehrazma, 2017). Telocytes may also contribute to the formation of reactive stroma since CD34-positive fibroblast-like cells were detected in the breast cancer stroma (Hua, Yu, Huang, Liao, & Xian, 2011), a possibility corroborated by our data, which indicate the association between telocytes and angiogenesis in the mammary gland; however, further experiments will be needed to assess the exact role of these cells in cancer.

5 | CONCLUSION

In general, the present study demonstrated the presence of telocytes in the perialveolar region during postlactational involution, as well as showed evidence that indicates a possible role of telocytes in extracellular matrix digestion through the synthesis of MMP9, which occurs concomitantly with the collapse of the alveoli. The study also showed that telocytes secrete VEGF and possibly contribute to angiogenesis during the involution of the gland. In conclusion, this study reiterates evidence that telocytes are a distinct cell type common to the stroma of several organs; they are therefore not data artifacts or misinterpretations of other cell types, such as prelymphatic endothelial cells. In addition, the present study demonstrates possible functions for these cells in the mammary gland morphophysiology.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

B. D. A. S. and J. S. M. contributed equally to this study. All authors (B. D. A. S., E. C. R. L., J. S. M., G. H. T., C. N. B., S. R. F., R. M. G., P. S. L. V., and S. R. T.) contributed to the design and interpretation of results and the revision of the manuscript. B. D. A. S., E. C. R. L., J. S. M., G. H. T., C. N. B., and S. R. F. performed the experiments. B. D. A. S. wrote the manuscript, R. M. G., P. S. L. V., and S. R. T. performed the final review of the manuscript.

ORCID

Patricia S. L. Vilamaior http://orcid.org/0000-0001-9559-5497
Sebastião R. Taboga http://orcid.org/0000-0002-0970-4288

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