Chapter 4
Telocytes in Chronic Inflammatory and Fibrotic Diseases

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Abstract Telocytes are a peculiar stromal (interstitial) cell type implicated in tissue homeostasis and development, as well as in the pathophysiology of several disorders. Severe damage and reduction of telocytes have been reported during fibrotic remodeling of multiple organs in various diseases, including scleroderma, Crohn's disease, ulcerative colitis, and liver fibrosis, as well as in chronic inflammatory lesions like those of primary Sjögren's syndrome and psoriasis. Owing to their close relationship with stem cells, telocytes are also supposed to contribute to tissue repair/regeneration. Indeed, telocytes are universally considered as "connecting cells" mostly oriented to intercellular signaling. On the basis of recent promising experimental findings, in the near future, telocyte transplantation might represent a novel therapeutic opportunity to control the evolution of chronic inflammatory and fibrotic diseases. Notably, there is evidence to support that telocytes could help in preventing abnormal activation of immune cells and fibroblasts, as well as in attenuating the altered matrix organization during the fibrotic process. By targeting telocytes alone or in tandem with stem cells, we might be able to promote regeneration and prevent the evolution to irreversible tissue injury. Besides exogenous transplantation, exploring pharmacological or non-pharmacological methods to enhance the growth and/or survival of telocytes could be an additional therapeutic strategy for many disorders.

4.1 Introduction

During both development and reparative processes of tissues and organs, the stromal compartment takes center stage not only by providing mechanical support and protection to parenchymal cells but also as a pivotal regulator of different cell activities, including proliferation, survival, differentiation, and metabolism [1, 2]. Accordingly,

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abnormalities in the stromal compartment may deeply impair tissue homeostasis, thus representing a key step in the development and progression of multiple pathologic conditions, such as chronic inflammatory, fibrotic, and neoplastic diseases.

It is now well established that the development and perpetuation of chronic inflammation are the consequence of a complex interplay between immune cells and nonimmune, tissue-resident, stromal (interstitial) cells. Actually, stromal cells are no longer believed to be "innocent bystanders" as a growing evidence suggests that they are active players in the induction and maintenance of the inflammatory process [3, 4]. For instance, stromal cells isolated from an inflammatory microenvironment are capable to drive the migration of immune cells in vitro [5]. In addition, under injury conditions tissue-resident stromal cells may secrete a variety of soluble mediators, including cytokines and chemokines that allow the transformation of diffuse immune infiltrates into highly organized tertiary lymphoid structures, namely, germinal center-like structures [6–8]. Nevertheless, chronic inflammation may often evolve into fibrosis, a condition also characterized by profound changes in the stromal compartment leading to progressive destruction of the normal tissue architecture and consequent organ dysfunction [9]. As far as the fibrotic process is concerned, fibroblasts are considered the principal effector stromal cells, as their chronic dysregulation may result in resistance to proapoptotic stimuli, increased transition to myofibroblasts, and excessive production and deposition of collagens and other extracellular matrix (ECM) components [10, 11].

Indeed, "classical" stromal cells include fibroblasts, myofibroblasts, dendritic cells, macrophages, vascular endothelial cells, and pericytes among others. In this context, stromal cells bearing very long cellular extensions have been long neglected and simplistically labeled as fibroblasts. However, in recent years, this view has rapidly changed because of the identification of an additional type of stromal cells with peculiar phenotypic features in a variety of human and animal tissues and organs [12–14]. These cells, named telocytes (telos, i.e., provided with long-distance cell projections), display a small cell body and extremely long and thin prolongations, termed "telopodes," and appear definitely distinct from the "classical" fibroblasts [12–16]. "Cells with telopodes" is the shortest definition of telocytes [14]. Telopodes typically exhibit a moniliform aspect characterized by the alternation of thin segments (podomers) and small dilated regions (podoms) accommodating the mitochondria, endoplasmic reticulum, and caveolae [12–14]. This peculiar ultrastructural phenotype observed under transmission electron microscopy (TEM) is currently considered as the most reliable hallmark for the identification of telocytes, which do not possess unique immunophenotypic characteristics [13, 14]. Although different markers have been proposed, at present a combination of CD34 and platelet-derived growth factor receptor α (PDGFRα) seems the best available choice for the immunohistochemical identification of telocytes under light and fluorescence microscopy (Fig. 4.1) [13, 14, 17–19]. Indeed, by immunoelectron microscopy, it could be demonstrated that the CD34-positive interstitial cells are ultrastructurally identifiable as telocytes (Fig. 4.1) [20]. A strong expression of CD34 and PDGFRα antigens has been firmly reported in telocytes from different organs [13, 14, 17–29]. Conversely, other markers resulted in

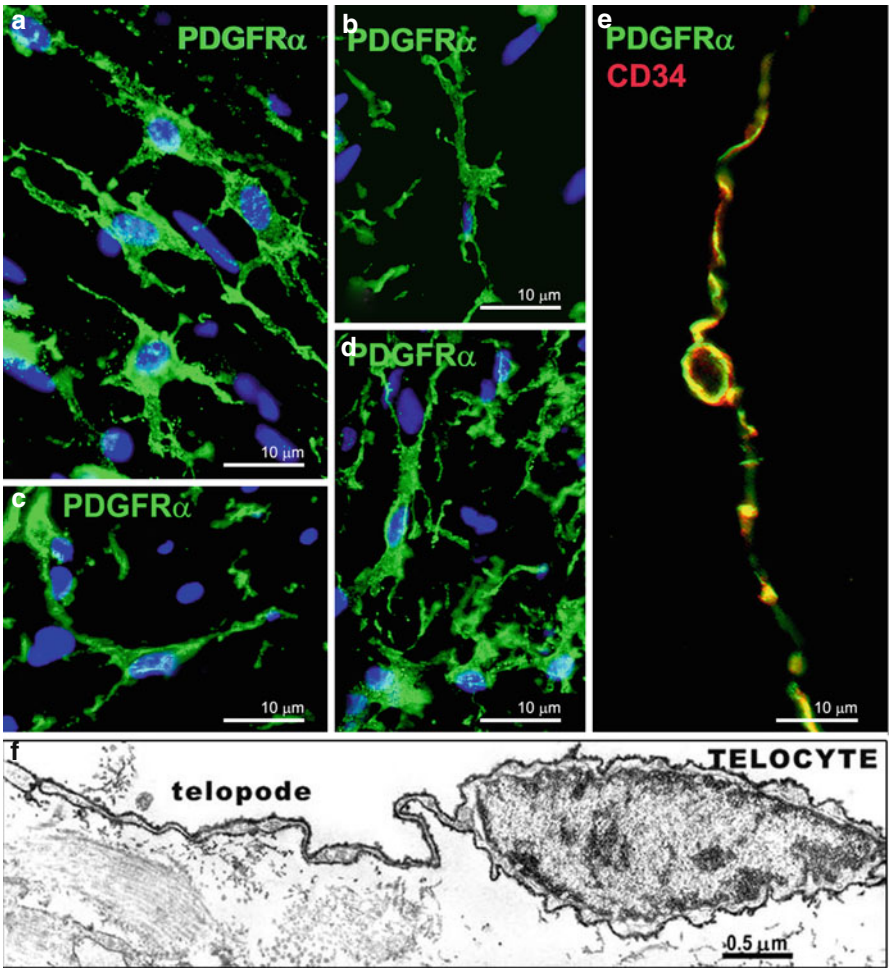

Fig. 4.1 Morphology and immunophenotype of telocytes in the human gastrointestinal tract. (**a**–**d**) PDGFRα-immunoreactivity (nuclei are blue stained with DAPI); (**e**) PDGFRα/CD34 double labeling; (**f**) CD34-immunoelectro-labeling. (**a**, **b**) Muscle layers (small intestine). Intramuscular PDGFRα-positive telocytes display two long telopodes and several short processes starting from the nucleated portion. (**c**) Submucosa (small intestine). PDGFRα-positive telocytes show a triangular body and three long and varicose telopodes. (**d**) Myenteric plexus region (small intestine). PDGFRα-positive telocytes display an oval body and several telopodes running in every direction. (**e**) A PDGFRα/CD34-positive telocyte at the border of a circular muscle bundle (small intestine) shows a small nucleated body and two long and thin telopodes starting from the opposite poles of the cell and with podomers and podoms clearly identifiable. (**f**) CD34-immunoelectro-labeling is present on the surface of a telocyte in the small intestine. The labeling appears as an electron-dense material distributed all along the plasma membrane, from which spherules protrude outside (Adapted with permission from [18, 20])

a weakly and inconstantly positive immunostaining of telocytes [13, 24]. Besides their ultrastructural and immunophenotypic features, a growing number of studies suggest that telocytes possess gene expression and proteomic profiles, as well as microRNA signature, that are definitely distinct from those of "classical" fibroblasts [14, 30–38].

According to their location in different organs and tissues, telocytes have been proposed to exert different functions and participate in a wide range of physiological processes. By their extremely long, tortuous, and overlapping telopodes, these cells interconnect to form a three-dimensional network that may function as a scaffold to define the correct tissue organization during prenatal life or repair/renewal in postnatal life, thus making a substantial contribution to the maintenance of local tissue homeostasis [13, 14, 18, 39, 40]. For instance, their importance in organ morphogenesis is supported by the evidence that during mouse heart development, telocytes act as mediators for heart compaction from embryonic myocardial trabeculae [41]. In different organs, telocytes occupy a strategic position in relation to stem cell niches, blood capillaries, and nerve bundles [14, 18, 22, 23, 26, 27, 42–46]. Telopodes also establish heterocellular contacts with other cell types including mast cells, basophils, lymphocytes, plasma cells, macrophages, or fibroblasts and noncellular elements, such as collagen and elastic fibers [14, 47, 48]. Furthermore, it appears that telocytes may participate in intercellular signaling not only by cell-to-cell contacts but also in a paracrine manner via the release of at least three different types of extracellular vesicles, namely, exosomes, ectosomes, and multivesicular cargos [14, 26, 49–54]. These vesicles might function as intercellular shuttles for the transfer of biological signals, including microRNAs, to neighboring cells [14]. Thus, differently from fibroblasts which, functionally, are mainly involved in the synthesis of collagen and other ECM components, it is believed that telocytes act as "connecting cells" being mostly oriented to intercellular signaling. As recently proposed, telocytes might even be considered as active players in immunomodulation and immune surveillance, acting like "local data suppliers" for the immune response [14, 28, 46, 47]. Increasing evidence also suggests that telocytes might act as "nurse" cells for adjacent tissue-resident stem cells and cooperate with them to promote tissue regeneration and/or repair [14, 21, 36, 44, 49, 55]. Moreover, telocytes have been suggested to participate in a variety of processes, such as stimulation of angiogenesis, inhibition of oxidative stress, and prevention of cellular aging [14, 34, 35]. An electrophysiologic activity of telocytes has also been demonstrated in organs like the myometrium and the heart [14, 56, 57]. Finally, in the gastrointestinal tract, telocytes have been proposed to participate in the regulation of neurotransmission and gut motility, presumably by spreading the slow waves generated by the pacemaker interstitial cells of Cajal (ICC) [13, 14, 18, 20].

Owing to the aforementioned intriguing roles proposed for telocytes, their possible involvement in different pathologic processes is being increasingly investigated [14, 22, 23, 27–29, 58–66]. In this regard, the present chapter will focus on the most recent findings concerning the implication of telocytes in a variety of chronic inflammatory, autoimmune, and fibrotic diseases.

4.2 Telocytes in Systemic Sclerosis: A Prototypic Multisystem Fibrotic Disorder

Systemic sclerosis (SSc), or scleroderma, is a chronic connective tissue disease characterized by extensive microvascular injury, immune system dysregulation, and progressive fibrosis affecting the skin and a variety of internal organs, especially the lungs, heart, and gastrointestinal tract [67, 68]. Endothelial cell damage/activation is supposed to be the initial event which together with inflammatory and autoimmune reactions leads to the chronic activation and transdifferentiation of fibroblasts into myofibroblasts, finally resulting in a deregulated wound healing process and severe tissue fibrosis with consequent multiple organ failure [67–69]. Indeed, visceral organ fibrosis is responsible for significant morbidity and is also a major cause of death in patients with SSc. Moreover, the concomitant fibroproliferative vasculopathy, characterized by subendothelial deposition of ECM in small and medium-sized arteries and arterioles, as well as the progressive loss of peripheral microvessels may lead to chronic tissue ischemia, clinically manifesting as digital ulceration and gangrene [70].

Two different clinical subsets of SSc are commonly recognized: limited cutaneous SSc (lcSSc) and diffuse cutaneous SSc (dcSSc), which differ in the extent of skin fibrosis, internal organ involvement, autoantibodies, prognosis, and survival [68]. In the early stages of both SSc subsets, the main cutaneous histopathological features are represented by perivascular inflammatory infiltrates, dermal edema, and a variable extent of ECM accumulation in the papillary and reticular dermis [67, 71]. Conversely, in advanced disease, severe dermal fibrotic changes with tightly packed and irregularly distributed collagen bundles, loss of capillaries, occlusion of arterioles, damage of nerve fibers, and atrophy of skin appendages are commonly observed [67, 70–72]. As far as internal organ involvement is concerned, the hallmark pulmonary histopathological lesion is nonspecific interstitial pneumonia characterized by cellular inflammation and fairly uniform interstitial fibrosis, which manifests as progressive thickening of the alveolar septa, ultimately resulting in alveolar airspace obliteration with consequent restrictive lung disease [73]. Cardiac manifestations include myocardial fibrosis, hypertrophy, and disorders of the coronary and conduction systems that can lead to congestive heart failure, arrhythmias, and sudden cardiac death [74]. Gastrointestinal involvement is commonly found in up to 90 % of SSc patients [75] and is characterized by fibrotic lesions in the muscularis mucosae, submucosa, and muscle layers together with smooth muscle cell atrophy [76, 77]. Clinically, gastrointestinal tract dysmotility is a major visceral manifestation, ranging from an asymptomatic form to severe paresis [75].

In SSc, fibroblasts are considered the principal effector cells [69]. In fact, it is well known that these stromal cells are chronically activated by a number of profibrotic cytokines, growth factors, and stimulatory autoantibodies and that they transform into apoptosis-resistant myofibroblasts which produce an excessive amount of collagens and other ECM components [67–69]. Abnormal SSc fibroblasts are believed to develop from a subset of cells that have escaped from normal control

mechanisms. Indeed, fibroblasts from clinically affected SSc skin still continue to produce excessive amounts of ECM proteins in vitro, suggesting that once activated, these cells establish a constitutive self-activation system [67, 78]. Moreover, there is substantial evidence that vascular endothelial cells, pericytes, and cells of both the innate and adaptive immune systems may contribute to abnormal fibroblast activation and fibrosis in SSc [69, 78, 79].

The recent identification of telocytes as a distinct stromal cell population of human dermis, where they have been proposed to participate to skin homeostasis, remodeling, and regeneration [21, 48], prompted us to investigate the possible involvement of this new interstitial cell type in the pathophysiology of SSc. By an integrated immunohistochemical and ultrastructural approach, we have recently shown for the first time that telocytes display ultrastructural damages, are significantly reduced, and progressively disappear from SSc skin lesions [22]. In normal skin, telocytes are organized to form three-dimensional networks with their telopodes distributed among collagen bundles and elastic fibers throughout the whole dermis (Fig. 4.2). Moreover, telocytes appear concentrated to surround microvessels (Fig. 4.2), nerves, hair follicles, and sebaceous and eccrine sweat glands. As far as SSc skin is concerned, the reduction in telocytes evolves differently according to disease subsets and stages (Fig. 4.3). In particular, in early lcSSc, telocytes are absent from the papillary dermis and reduced in some areas of the reticular dermis. In advanced lcSSc, the loss of telocytes is severe also in the reticular dermis and the connective tissue surrounding skin adnexa. On the contrary, in the early stage of dcSSc, which is characterized by a more rapid disease progression [67], telocytes are very few or absent in both the papillary and reticular dermis, and they almost completely disappear in advanced dcSSc skin lesions (Fig. 4.3). Thus, the progression in telocyte reduction occurs earlier and is more severe in dcSSc than in lcSSc. Interestingly, such a reduction occurs in parallel with the severity of telocyte ultrastructural abnormalities, including swollen mitochondria, cytoplasmic vacuolization, and presence of lipofuscinic bodies, which suggest a cellular degenerative process already present in the early stage of dcSSc and more marked in the advanced stage of both disease subsets (Figs. 4.4 and 4.5). In addition, telocytes often establish intercellular contacts with inflammatory and immune cells in early SSc skin lesions.

Different mechanisms have been proposed to be responsible for the damage and loss of telocytes in clinically affected SSc skin [22]. For instance, it has been suggested that the chronic ischemic microenvironment of fibrotic skin, characterized by low oxygen levels, generation of reactive oxygen species, and scarcity of nutrients, may compromise the telocyte metabolism, thus provoking profound cell sufferance. In fact, in SSc dermis the reduction in telocytes seems to be paralleled by the reduction in microvessels. The hypothesis of an ischemic injury is mainly supported by the presence of telocytes with numerous swollen mitochondria and extensive cytoplasmic vacuolization. Of note, the most severely affected telocytes appear to be those embedded in the fibrotic ECM and those surrounding occluded microvessels, while the telocytes found around patent microvessels still display a normal morphology, even in the advanced disease stages [22]. With disease progression, the more severe and extended damage of telocytes observed under TEM might be

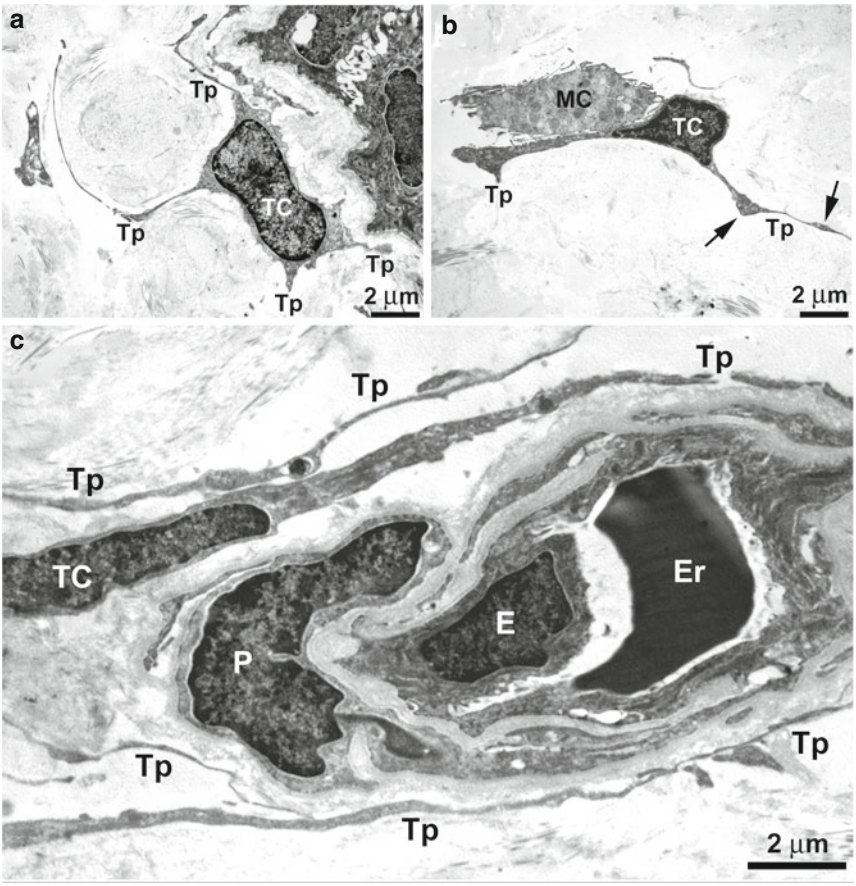

Fig. 4.2 Telocytes in normal skin (transmission electron microscopy). (**a**–**c**) In both the papillary dermis (**a**) and reticular dermis (**b**, **c**), telocytes have a small cell body and very long and thin processes (telopodes) that are collagen embedded or lining elastic fibers. Telocytes lack a basal lamina and have a scarce cytoplasm surrounding the nucleus with few mitochondria and cisternae of the endoplasmic reticulum and a small Golgi apparatus. Telopodes display a moniliform aspect due to the alternation of thin segments (podomers) and dilated segments (podoms) oval or triangular in shape (**b**, *arrows*). (**b**) A telocyte is in contact with a mast cell. (**c**) Telocytes are closely associated with each other. The telopodes of perivascular telocytes encircle the basal lamina of a blood microvessel; pericytes are embedded in the vessel basal lamina (**c**). *TC* telocyte, *Tp* telopode, *MC* mast cell, *E* endothelial cell, *Er* erythrocyte, *P* pericyte (Adapted with permission from Manetti et al. [22])

caused by their entrapment in a poorly permeable ECM due to the overproduction and accumulation of abnormal collagen and elastic fibers by activated fibroblasts/myofibroblasts. Finally, when considering the autoimmune background of SSc [67, 68], the possibility that telocytes might be important/specific target cells of autoantibodies was also taken into account. However, this latter hypothesis seems little supported by the fact that telocytes display a normal morphology in clinically non-involved skin biopsies from SSc patients.

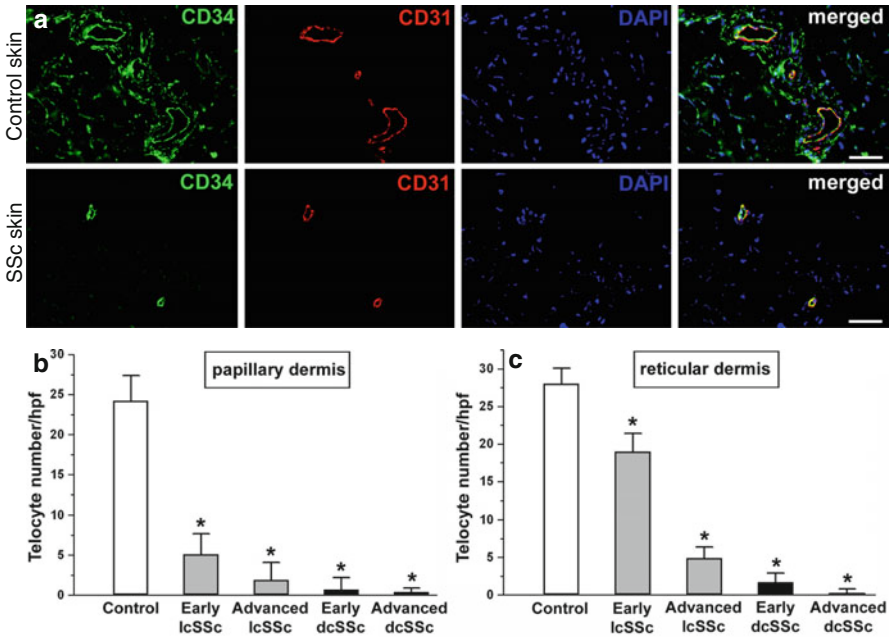

Fig. 4.3 Quantitative analysis of telocytes in skin sections from controls and patients with systemic sclerosis (*SSc*) double immunolabeled for CD34 (*green*) and CD31 (*red*) and counterstained with DAPI (*blue*) for nuclei. (**a**) Representative photomicrographs from control and clinically affected SSc skin samples are shown. Telocytes are identified as CD34-positive/CD31-negative spindle-shaped cells, while microvessels are CD34/CD31 double positive. (**b**, **c**) Telocytes are reduced in both the papillary and reticular dermis of SSc patients throughout different disease stages. Data are represented as mean ± SD. $*P<0.05$ vs control. *lcSSc* limited cutaneous SSc, *dcSSc* diffuse cutaneous SSc (Adapted with permission from Manetti et al. [22])

In a subsequent study, these findings were further extended by the evidence that in SSc, the loss of telocytes is not restricted to the skin, but it is a widespread process affecting multiple visceral organs targeted by the fibrotic process, such as the gastric wall, the myocardium, and the lung (Figs. 4.6 and 4.7) [29].

According to the numerous functions proposed for telocytes [14], several hypotheses have been formulated on the possible pathophysiologic implications that their damage and systemic loss might have in SSc [22, 29]. It has been suggested that by their long telopodes, telocytes might act as supporting cells and form a scaffold to guide the migration of other cells and the correct ECM assembly, thus contributing to define the correct spatial organization of tissues and organs [13, 40, 41]. Indeed, in the stromal compartment of many organs, telopodes are usually collagen embedded or lining elastic fibers. Interestingly, in SSc skin some telocytes were found to surround with their telopodes very large and abnormal aggregates of elastin and collagen fibers, likely in the attempt to limit their spreading into the interstitium. Therefore, it is conceivable that the loss of telocytes could mechanically contribute to the altered three-dimensional organization of the ECM within the stromal

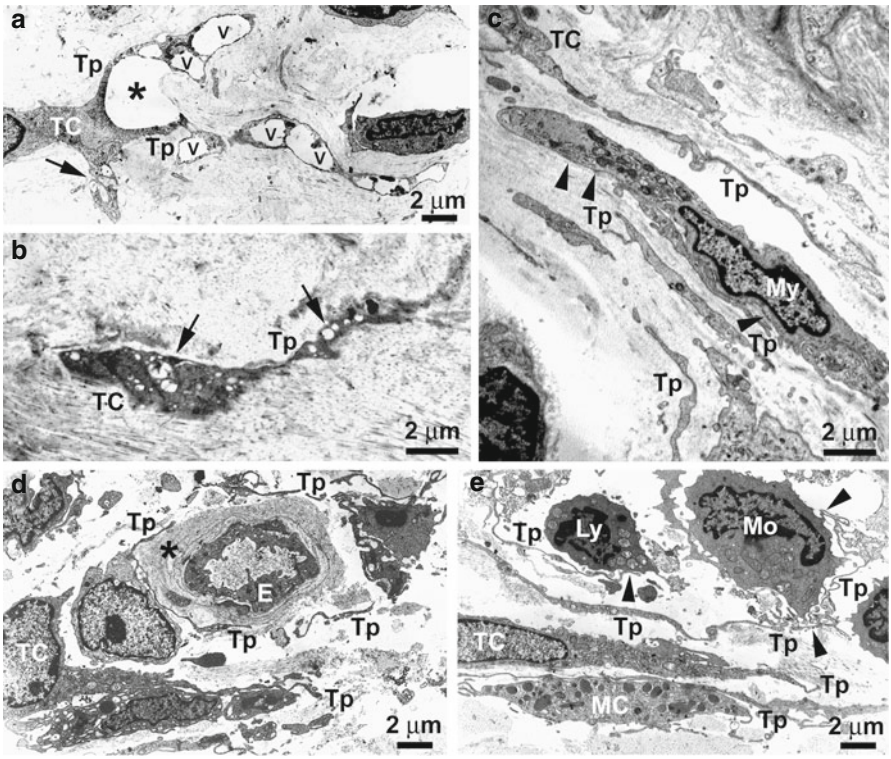

Fig. 4.4 Telocytes in limited cutaneous systemic sclerosis (*lcSSc*) skin (transmission electron microscopy). (**a**) Early lcSSc. A telocyte displaying an enlarged shape, due to the presence of large vacuoles (*v*) in its telopodes, surrounds an area of dermal edema (*asterisk*). Both normal mitochondria and swollen mitochondria with a clear matrix and few cristae (*arrow*) are identifiable in the cytoplasm. (**b**) Advanced lcSSc. A degenerating telocyte entrapped in the fibrotic extracellular matrix shows numerous swollen mitochondria (*arrows*). The cytoplasm is dark and contains vacuoles and lipofuscinic bodies. (**c**) Early lcSSc. Telocytes and telopodes displaying a normal morphology are present in the close vicinity of or even in contact with a myofibroblast which shows a large body rich in rough endoplasmic reticulum, mitochondria, and myofilaments. Subplasmalemmal focal densities are evident (*arrowheads*). (**d**) Early lcSSc. Some telocytes and telopodes with a normal morphology are present around a blood vessel displaying a patent lumen. The vessel basal lamina is markedly thickened (*asterisk*). (**e**) Early lcSSc. Normal telocytes with very long and convoluted telopodes surround a perivascular inflammatory infiltrate composed of monocytes and lymphocytes. Telopodes establish cell-to-cell contacts with inflammatory cells (*arrowheads*). A mast cell is also in contact with telopodes. *TC* telocyte, *Tp* telopode, *My* myofibroblast, *E* endothelial cell, *Ly* lymphocyte, *Mo* monocyte, *MC* mast cell (Reproduced with permission from Manetti et al. [22])

compartment of any organ undergoing fibrotic remodeling, such as the skin, the gastric wall, the myocardium, and the lung [22, 29]. During the fibrotic process, the loss of telocytes might even favor the uncontrolled activation of fibroblasts and their transition to profibrotic myofibroblasts. In fact, telocytes may convert the interstitium into an integrated system that contributes to the maintenance of organ homeostasis.

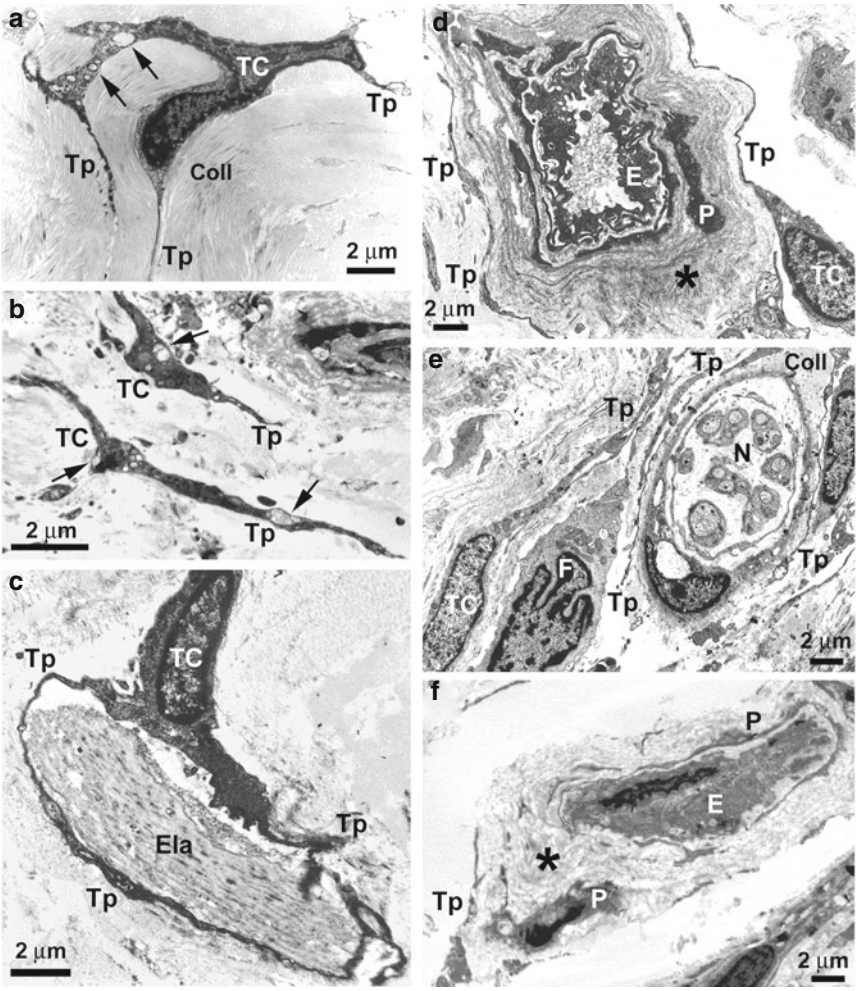

Fig. 4.5 Telocytes in diffuse cutaneous systemic sclerosis (*dcSSc*) skin (transmission electron microscopy). (**a**) Early dcSSc. A telocyte with a small perinuclear cytoplasm and slender telopodes is embedded in a matrix composed of closely packed collagen bundles. Swollen mitochondria and vacuoles (*arrows*) are present in the cytoplasm. (**b**) Advanced dcSSc. Telocytes and telopodes embedded in the fibrotic extracellular matrix show features of degenerating cells. The cytoplasm is dark and contains swollen mitochondria (*arrows*), vacuoles, and lipofuscinic bodies. Many cell debris are evident. (**c**) Early dcSSc. A telocyte displaying a normal morphology embraces with telopodes a large and abnormal elastin fiber. (**d**) Early dcSSc. Normal telocytes surround the thickened basal lamina (*asterisk*) of a blood vessel displaying a patent lumen. (**e**) Early dcSSc. Telocytes with a normal morphology are evident around nerve bundles. Abundant collagen fibers separate telopodes from the nerve bundle. A fibroblast is in the close vicinity of a telocyte and is surrounded by telopodes. (**f**) Advanced dcSSc. Telocytes are not identifiable around an occluded microvessel. Only a few cell debris are observed. The vessel basal lamina is markedly thickened (*asterisk*). *TC* telocyte, *Tp* telopode, *Coll* collagen, *Ela* elastin, *E* endothelial cell, *P* pericyte, *N* nerve, *F* fibroblast (Reproduced with permission from Manetti et al. [22])

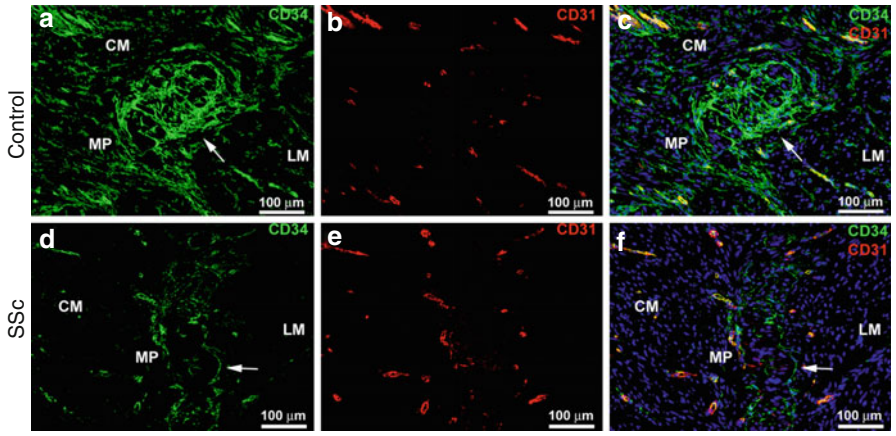

Fig. 4.6 Gastric wall specimens from controls (**a**–**c**) and patients with systemic sclerosis (*SSc*) (**d**–**f**). (**a**–**f**) Double immunofluorescence labeling for CD34 (*green*) and CD31 (*red*) with DAPI (*blue*) counterstain for nuclei. Telocytes are CD34 positive and CD31 negative, while vascular endothelial cells are CD34/CD31 double positive. (**a**–**c**) In control gastric wall, telocytes form a network around smooth muscle bundles and cells in the circular and longitudinal muscle layers. At the myenteric plexus, telocytes form a complex network enveloping the ganglia (*arrow*) and the nerve strands in the interganglionic region. Telopodes appear intermingled with ganglion cells. (**d**–**f**) In SSc gastric wall, telocytes are not present in the fibrotic areas of muscle layers. The network of telocytes is discontinuous or even almost completely absent around myenteric plexus ganglia (*arrow*) and nerve strands. *CM* circular muscle layer, *LM* longitudinal muscle layer, *MP* myenteric plexus (Reproduced with permission from Manetti et al. [29])

In particular, these cells seem to be involved in intercellular signaling, either directly by intercellular contacts or indirectly by shedding microvesicles and exosomes or secreting paracrine signaling molecules, including microRNAs [14, 26, 49–54]. Interestingly, intercellular contacts between telocytes and fibroblasts or myofibroblasts have been described in different organs [21, 22, 46, 48], and thus it is tempting to speculate that telocytes could be involved in the maintenance of local tissue homoeostasis by controlling fibroblast/myofibroblast activity. In SSc skin and visceral organs, this control is likely impaired because of the progressive reduction and loss of telocytes [22, 29].

Another attractive hypothesis is that the disappearance of telocytes could impair stem cell-mediated tissue regeneration. Indeed, there is substantial evidence that in several organs, such as the skin, the heart, and the lung, telocytes might cooperate with tissue-resident stem niches to promote regeneration and/or repair [14, 21, 26, 44, 80]. In support to this hypothesis, we and others observed telocytes surrounding stem cell niches in the normal skin [21, 22], but they were rarely seen in affected SSc skin. Furthermore, vascular wall-resident stem cell niches could not be detected in most severely affected skin biopsies, suggesting that telocyte loss might contribute to the depletion of functional stem cell niches with consequent impairment of skin regeneration and/or repair in SSc patients [22].

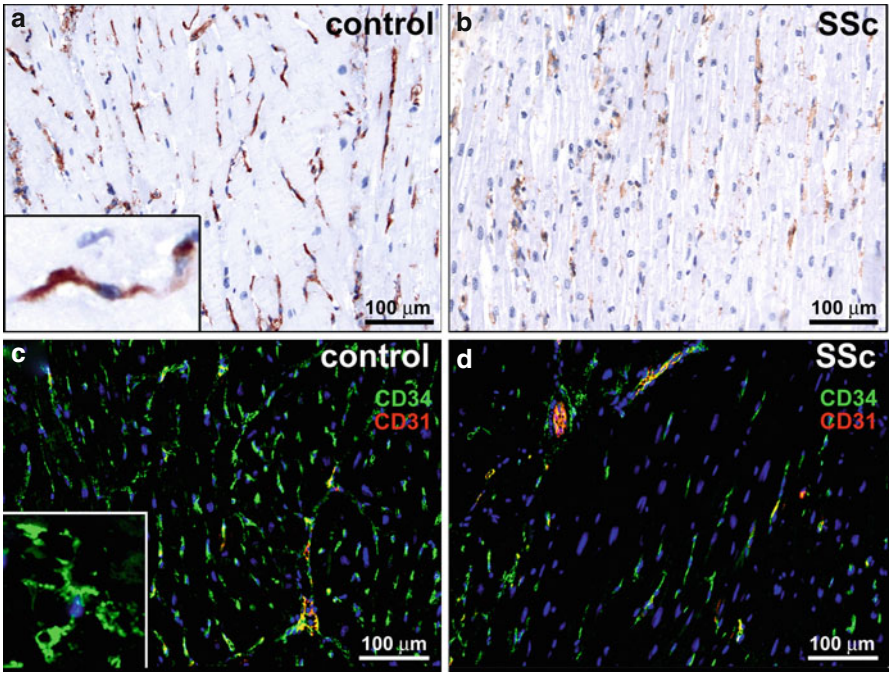

Fig. 4.7 Left ventricular myocardium specimens from controls (**a**, **c**) and patients with systemic sclerosis (*SSc*) (**b**, **d**). (**a**, **b**) CD34 immunoperoxidase labeling with hematoxylin counterstain. (**c**, **d**) Double immunofluorescence labeling for CD34 (*green*) and CD31 (*red*) with DAPI (*blue*) counterstain for nuclei. Myocardial telocytes are CD34 positive and CD31 negative, while vascular endothelial cells are CD34/CD31 double positive. (**a**, **c**) In control myocardium, numerous telocytes are located in the interstitium surrounding the cardiomyocytes. *Insets*: At higher magnification view, myocardial telocytes display a small fusiform cell body with long processes placed between cardiomyocytes. (**b**, **d**) In the fibrotic areas of SSc myocardium, telocytes are almost completely undetectable (Adapted with permission from Manetti et al. [29])

In the myocardium, it has also been shown that telocytes and cardiomyocytes are directly connected and might represent a "functional unit," possibly mediating the electrical coupling of cardiomyocytes [24, 81]. Therefore, it is possible that in SSc heart, the loss of myocardial telocytes (Fig. 4.7) might even be implicated in the arrhythmogenesis and disturbances of the cardiac conduction system [24, 29, 74]. Finally, the loss of the telocyte network observed in the muscularis propria and myenteric plexus of SSc gastric wall (Fig. 4.6) might contribute to gastric dysmotility, clinically manifesting as delayed gastric emptying or gastroparesis [29, 75]. Indeed, in the gastrointestinal tract, telocytes have been proposed to play a role in the regulation of neurotransmission, possibly by spreading the slow waves generated by the ICC [13, 18, 20]. Interestingly, there is also evidence that in SSc, gastrointestinal tract dysmotility may be related to severe damage of the myenteric neural structures and a reduction in the ICC population [29, 77, 82].

4.3 Telocytes in Inflammatory Bowel Diseases

Inflammatory bowel diseases, including Crohn's disease (CD) and ulcerative colitis (UC), are complex disorders in which the interaction of genetic, environmental, and microbial factors drives chronic relapsing and remitting intestinal inflammation that finally leads to extensive tissue fibrosis [83–85]. This is particularly relevant for CD, which may affect the entire gastrointestinal tract with a prevalence of terminal ileum. Indeed, in UC the deposition of the ECM is mainly restricted to the mucosal and submucosal layers of the large bowel, while in CD, fibrosis commonly involves the entire bowel wall, including the mucosa, submucosa, muscularis propria, and subserosa layers, and can result in critical narrowing of the lumen and strictures or stenosis, leading to intestinal obstruction that requires surgery [86]. Accordingly, the frequency of benign stenosis in UC is much lower than in CD, reported as being 3.2–11.2 %, with fibrosis in the submucosa or deeper pointed out as one of the causes [87]. However, increasing evidence indicates that the development of intestinal fibrosis in UC is a neglected problem which has remained largely unexplored [88].

In inflammatory bowel diseases, fibrosis closely follows the distribution and location of inflammation [83, 86, 89]. Of note, there is evidence that chronic exposure of intestinal fibroblasts to inflammatory mediators may drive their transition to activated α-smooth muscle actin (α-SMA)-expressing myofibroblasts, with consequent abnormal collagen production and tissue remodeling [89]. However, it also appears that inflammation subsequently plays a minor role in fibrosis progression, and, accordingly, anti-inflammatory treatment may not be able to limit intestinal fibrosis once excessive ECM deposition has started [86]. Progressive intestinal wall fibrosis ultimately results in a stiff intestine unable to carry out peristalsis, contributing to the abdominal pain and diarrhea commonly experienced by patients with active disease or even in remission. In fact, bowel dysmotility has been well established in both CD and UC [83–85].

Motility of the gastrointestinal tract involves complex processes that require the structural integrity and functionality of different cellular elements. Enteric neurons and glial cells, together with ICC, represent the main regulators of motor functions in the gut wall, ensuring coordinated patterns of smooth muscle cell activity [90, 91]. In particular, the ICC are considered the pacemaker cells and the principal mediators of gut neurotransmission [92]. This complex neural/myogenic network appears to be markedly altered in CD and UC patients, as demonstrated by severe damages of the enteric neural and glial structures as well as a marked reduction in the number of ICC [93–96].

However, in recent years it became evident that also telocytes might be part of this neural/myogenic network. Indeed, it could be demonstrated that both in muscle layers and at the myenteric plexus, telocyte processes form networks intermingling with those of ICC, suggesting that these two cell types might establish cell-to-cell contacts [18]. In particular, since a subset of intramuscular telocytes and ICC seem to be part of a unique network, in which the latter are preferentially in close contact with nerve endings, it has been proposed that telocytes might participate in the

regulation of gastrointestinal motility, presumably contributing to the spreading of the slow waves generated by the ICC [13, 18]. Within the gastrointestinal neuromuscular compartment, ICC and telocytes can be easily distinguished on the basis of their different immunophenotypes [18]. In fact, the ICC are positive for c-kit (CD117) and negative for CD34 and PDGFRα [18], while telocytes are positive for CD34 and PDGFRα and negative for c-kit [18]. It has also been ascertained that in the gastrointestinal tract, telocytes correspond to the cells formerly identified as CD34-positive interstitial cells or PDGFRα-positive "fibroblast-like" cells and implicated in the enteric neurotransmission [18, 20, 97, 98].

Considering that gastrointestinal dysmotility with ICC defects is a peculiar pathological feature of both CD and UC [93–96, 99] and that ICC and telocytes are in close relationship within the gut neuromuscular compartment [18], we recently carried out two different studies in which we investigated the presence and distribution of telocytes in surgical specimens obtained from the terminal ileum of CD patients and the colon of UC patients [23, 27].

In CD, the most peculiar histopathological features of the affected intestinal wall segments are represented by discontinuous signs of inflammation and fibrosis, also referred to as "skip lesions." Interestingly, in disease-unaffected specimens from CD patients, telocytes display a distribution similar to control specimens, showing a slender-nucleated body with two or more telopodes and running parallel to each other and/or forming networks throughout the different ileal wall layers, from the mucosa to the subserosa [23]. Conversely, in sections from disease-affected specimens, telocytes disappear, particularly in areas displaying severe fibrosis and architectural derangement of the intestinal wall [23]. In the thickened muscularis mucosae of most severe cases, the few remaining telocytes are mainly located among smooth muscle bundles and cells. Some reactive lymphoid aggregates (e.g., granulomas), especially those surrounded by a prominent and diffuse inflammatory infiltrate, appear completely encircled by telocytes, likely in the attempt to limit their spreading in the connective tissue. Instead, telocytes almost completely disappear around lymphoid aggregates entrapped within the fibrotic tissue containing many α-SMA-positive myofibroblasts [23]. In the muscularis propria of disease-affected CD samples, characterized by a severe derangement of both the circular and longitudinal muscle layers, the telocyte network is preserved among smooth muscle bundles in some areas close to others where telocytes are completely absent. This severe architectural derangement involves also the myenteric plexus, where a discontinuous network of TC is present around ganglia and in the interganglionic region (Fig. 4.8) [23].

As far as UC is concerned, the presence and distribution of telocytes were investigated in full-thickness biopsies of the left colon obtained from UC patients categorized in an early phase or an advanced phase of fibrotic remodeling of the colonic wall [27]. In early fibrotic UC cases, fibrosis affects the muscularis mucosae and submucosa, while the muscularis propria is spared. In particular, the submucosa is characterized by the presence of areas displaying edema and a pattern of incoming fibrosis abruptly mixed with areas displaying established fibrosis with abundant and closely packed collagen bundles. In advanced fibrotic UC cases, an increased ECM deposition is found in the muscularis mucosae, which appears markedly thickened

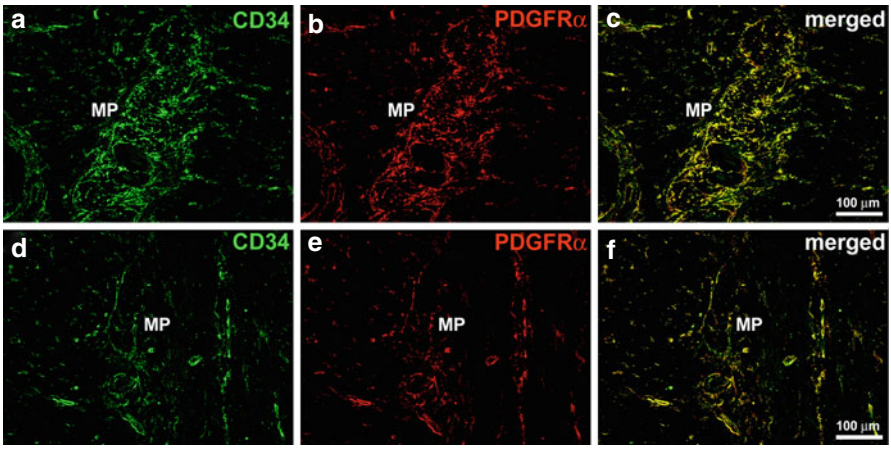

Fig. 4.8 (**a–c**) Control ileal specimens. (**d–f**) Affected ileal specimens from Crohn's disease (*CD*) patients. (**a–f**) Double immunofluorescence labeling for CD34 (*green*) and platelet-derived growth factor receptor α (PDGFRα) (*red*). In the ileal wall, all CD34-positive telocytes are also PDGFRα positive. (**a–c**) In control specimens, telocytes form a broad network surrounding the myenteric plexus. (**d–f**) In severely damaged areas of affected CD specimens, the telocyte network around the myenteric plexus is discontinuous or even completely absent. *MP* myenteric plexus (Reproduced with permission from Milia et al. [23])

and widespread in the submucosa. Moreover, fibrosis extends to involve also wide areas of the circular and longitudinal muscle layers and the myenteric plexus [27]. Interestingly, the fibrotic changes of the colonic wall seem to be paralleled by a severe reduction in telocytes. In fact, a significant reduction in telocytes is found in the muscularis mucosae and submucosa of both early and advanced fibrotic UC colonic wall. Conversely, while a normal distribution of telocytes is observed in the muscularis propria of early fibrotic UC, the network of telocytes is reduced or even completely absent in fibrotic areas of muscle layers and around myenteric ganglia of advanced fibrotic UC cases (Fig. 4.9) [27]. Of note, these data are closely consistent with those reported in "skip lesions" of disease-affected CD specimens, in which telocytes specifically disappear in areas displaying severe fibrosis and architectural derangement of the intestinal wall [23]. Finally, as revealed by CD34/c-kit double immunostaining, in the muscularis propria of both CD and UC, the disappearance of telocytes seems to be paralleled by the loss of the ICC network (Fig. 4.9) [23, 27].

As above discussed for SSc, the loss of telocytes might have different causes and pathophysiologic implications in CD and UC. We suppose that the fibrotic process may entrap telocytes in a poorly permeable ECM, with profound cell sufferance. Moreover, the excessive deposition of ECM and the progressive reduction in telocytes may alter the spatial relationships of telopodes with neighboring immune cells, fibroblasts, smooth muscle cells, ICC, and nervous structures, possibly impairing intercellular signaling and functions. However, whether the loss of telocytes might even precede the onset of fibrosis rather than being merely a consequence of the fibrotic process is difficult to be elucidated. In this context, the

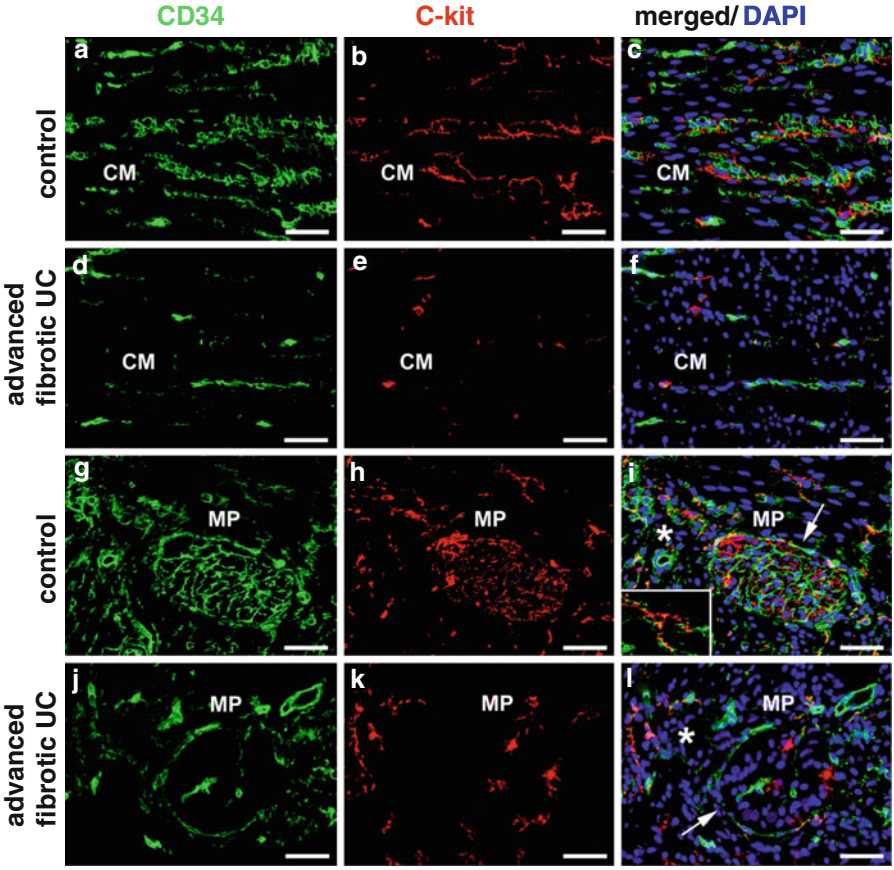

Fig. 4.9 Muscularis propria of colonic wall specimens from controls (**a–c** and **g–i**) and advanced fibrotic ulcerative colitis (*UC*) cases (**d–f** and **j–l**). (**a–l**) Double immunofluorescence labeling for CD34 (*green*) and c-kit/CD117 (*red*) with DAPI (*blue*) counterstain for nuclei. Telocytes are CD34 positive/c-kit negative, whereas interstitial cells of Cajal (*ICC*) are c-kit positive/CD34 negative. (**a–c**) In muscle layers of control colonic sections, telocytes and ICC form interconnected networks among smooth muscle bundles. (**d–f**) In muscle layers of advanced fibrotic UC cases, very few telocytes and ICC can be observed. Representative microphotographs of the circular muscle layer are shown. (**g–i**) Note the abundant networks of telocytes and ICC around ganglia (*arrow* in **i**) and in the interganglionic region (*asterisk* in **i**) of the myenteric plexus of control colonic wall. (*Inset* in **i**): Higher magnification view of an ICC surrounded by telopodes. (**j–l**) In advanced fibrotic UC cases, both telocytes and ICC are scarce around myenteric ganglia (*arrow* in **l**) and in the interganglionic region (*asterisk* in **l**). *CM* circular muscle layer, *MP* myenteric plexus (Reproduced with permission from Manetti et al. [27])

findings that in early fibrotic UC cases telocytes are already reduced in the edematous, less fibrotic areas of submucosa suggest that telocyte loss may be a very precocious event during fibrotic remodeling of the intestinal wall [27]. The progressive loss of telocytes in the intestinal wall could also contribute to the altered three-dimensional organization of the ECM and to the progression of fibrosis, eventually

favoring fibroblast-to-myofibroblast transition as proposed in the skin of SSc patients [22]. In support to this last hypothesis, in the colonic submucosa of UC patients, the disappearance of telocytes is paralleled by the increase in the number of α-SMA-positive myofibroblasts [27]. However, as proposed by other authors [17], we cannot completely rule out the possibility that during pathological processes, some telocytes might even change their immunophenotype (e.g., loss of CD34 expression and gain of other markers, such as α-SMA), thus contributing to the increase in the myofibroblast population. Nevertheless, we should consider that CD34/α-SMA double immunostaining did not reveal the presence of double-positive transitioning stromal cells in colonic sections from UC patients [27]. Furthermore, there is clear ultrastructural evidence of telocyte degeneration, rather than activation/transdifferentiation into myofibroblasts, in the setting of tissue fibrosis [22].

In the gut, the three-dimensional network of telocytes has also been proposed to play a specific mechanical and supporting role throughout the different bowel wall layers, being resistant to and deformable following intestine movements [13, 18, 20]. Even more importantly, in the muscularis propria telocytes and ICC may form interconnected networks surrounding smooth muscle bundles and myenteric plexus ganglia [13, 18, 23, 27]. Thus, it has been hypothesized that the reduction in both telocyte and ICC networks within the neuromuscular compartment of the intestinal wall might substantially contribute to gastrointestinal dysmotility in both CD and UC patients [23, 27].

4.4 Telocytes in Liver Fibrosis

Liver fibrosis, primarily as a result of chronic viral hepatitis and fatty liver diseases associated with obesity, is a worldwide burden [100]. Liver fibrosis can further progress to cirrhosis, representing a major cause of morbidity and mortality worldwide, and is responsible for several end-stage liver disease complications, including portal hypertension, impaired metabolic capacity, synthetic dysfunction, and ascites [100, 101]. Hepatic stellate cells are considered as the primary source of the fibrogenic population in the liver [102]. In addition, portal fibroblasts, bone marrow-derived cells, circulating fibrocytes, and fibroblasts deriving from epithelial-mesenchymal transition may also be implicated in the hepatic fibrogenic process [102, 103].

The existence of telocytes in the liver, particularly in the Disse space with a similar density in the four hepatic lobes, has been established [104]. Furthermore, the potential role of telocytes in liver regeneration has been emphasized in a mouse model of partial hepatectomy [105]. In a recent study, the possible involvement of telocytes in liver fibrosis has also been investigated [59]. In particular, it has been reported that telocytes are reduced in the human fibrotic liver, further supporting that within the stromal hepatic compartment telocytes are different from hepatic stellate cells which, instead, are well known to be increased during liver fibrosis [59]. Taking into account the “connecting cell” function of telocytes, the authors suggested that they might be able to control the activity of hepatic stellate cells.

Therefore, telocyte loss might contribute to hepatic stellate cell dysregulation in the fibrotic liver [59]. In addition, the disappearance of telocytes might impair hepatocytes and stem cell-mediated liver regeneration. Indeed, it has been shown that telocytes have a close spatial relationship with hepatic putative stem cells and that they may influence proliferation of hepatocytes and/or the activation of hepatic stem cells [105]. Thus, in human liver fibrosis, the reduction in telocytes might contribute to the depletion of stem cell niches or hepatocyte dysfunction and impair liver regeneration/repair.

4.5 Telocytes in Primary Sjögren's Syndrome

Primary Sjögren's syndrome (pSS) is a systemic autoimmune disorder mainly affecting women during the fourth and fifth decades of life and characterized by chronic inflammation of exocrine glands leading to progressive functional impairment [106, 107]. The histological hallmark of pSS is a focal lymphocytic sialadenitis, and the presence of at least one focus (i.e., an aggregate of at least 50 lymphocytes and plasma cells) in 4 mm^2 of minor salivary gland (MSG) tissue allows the diagnosis of pSS [108]. In the last decades, the pathogenic role of stromal cells in systemic inflammatory/autoimmune disorders including pSS has been extensively investigated [3, 4]. In this context, the unique phenotype, ultrastructural characteristics, tissue distribution, and multiple intercellular connections of telocytes, as well their putative role in local immune surveillance and homeostasis [14, 47], raised the possibility of their involvement in the pathogenesis of inflammatory/autoimmune disorders as pSS.

With regard to salivary glands, to date telocytes have been described in parotid glands and labial MSGs, where these peculiar stromal cells surround secretory and excretory structures, namely, acini and ducts, and are also in close contact with blood vessels [28, 109]. Interestingly, it has been recently highlighted a possible association between telocyte patterns and the extent of glandular inflammation and lymphoid organization in MSGs from patients with pSS [28]. Notably, telocytes are markedly reduced in MSGs of pSS patients with respect to normal and nonspecific chronic sialadenitis (NSCS; i.e., presence of scattered lymphocyte aggregates that do not reach the number of 50 and therefore cannot be classified as foci) MSGs, and such a decrease parallels the worsening of glandular inflammation and the progression of ectopic lymphoid neogenesis. Indeed, periductal telocytes are reduced in the presence of smaller inflammatory foci and completely absent in the presence of germinal center-like structures, thus closely reflecting disease severity [28]. In addition, while in other inflammatory pathological conditions like CD lymphoid aggregates/granulomas are entirely surrounded by telocytes, suggesting a certain attempt to control their spreading [23], the complete absence of telocytes around MSG inflammatory foci underscores that this potential protective mechanism may be impaired in pSS.

In keeping with the proposed function of telocytes as key tissue homeostasis regulators, their local loss might contribute to breaking out of the immune homeo-

stasis contributing to the pathogenesis of focal lymphocytic sialadenitis. This hypothesis is supported by the evidence that telocytes are markedly and specifically reduced in focal lymphocytic sialadenitis but not in NSCS. In this setting, the formation and maintenance of focal lymphocytic sialadenitis as well as the development of ectopic lymphoid structures during chronic inflammation are dependent on the expression of lymphotoxins, cytokines, and chemokines by several cell types [28]. Previous studies reported that in NSCS MSGs, the expression levels of several of these mediators are similar to that of normal MSGs, while they are significantly upregulated in pSS MSGs compared to both normal and NSCS MSGs [28]. These observations suggest that the peculiar inflammatory microenvironment of pSS MSGs might even be one of the causes of local telocyte damage and loss. Moreover, the fact that in pSS MSGs telocytes are preserved around the acini not affected by the inflammatory process allows to speculate that the cross talk between epithelial or infiltrating immune cells and telocytes occurs in a paracrine manner limited to each secretory unit [28]. However, whether the loss of telocytes in pSS may represent either the cause or the consequence of local inflammation remains still unknown. To further gain insights on the possible contribution of telocytes to pSS pathophysiology and the exact mechanisms underlying telocyte cross talk with other MSG cell types and their disappearance during focal lymphocytic sialadenitis, future ultrastructural and functional studies will be required.

4.6 Telocytes in Psoriasis

Psoriasis is a common inflammatory skin condition, mainly considered a keratinization disorder on a genetic background. Indeed, the dermis contribution to the pathogenesis of psoriasis is frequently eclipsed by remarkable epidermal phenomena.

Among stromal cells, several studies have focused on dendritic cells as major participants in the chronic skin inflammatory process that characterizes psoriasis [110, 111]. Besides dendritic cells, the ability of telocytes to establish intercellular communications (either physical or paracrine) with immune cells has been well documented in several organs, including the skin [14, 21, 22, 46, 47]. Thus, in the context of the vast immunology of psoriasis, it has been hypothesized that telocytes could be involved in disease initiation and/or progression [58]. Furthermore, substantial evidence indicates that angiogenesis may also contribute to the pathogenesis and clinical signs of psoriasis [112]. Noteworthy, previous studies have shown that telocytes may be involved in angiogenesis, providing their support in the reparatory process after acute myocardial infarction [50]. Therefore, the possible involvement of telocytes in psoriasis-related angiogenesis has also been investigated [58].

In their recent study, Manole et al. demonstrated a reduction in the number of telocytes in papillary dermis of psoriasis vulgaris, as well as a recovery of these cells after local corticoid therapy [58]. Of note, the density of telocytes with a normal

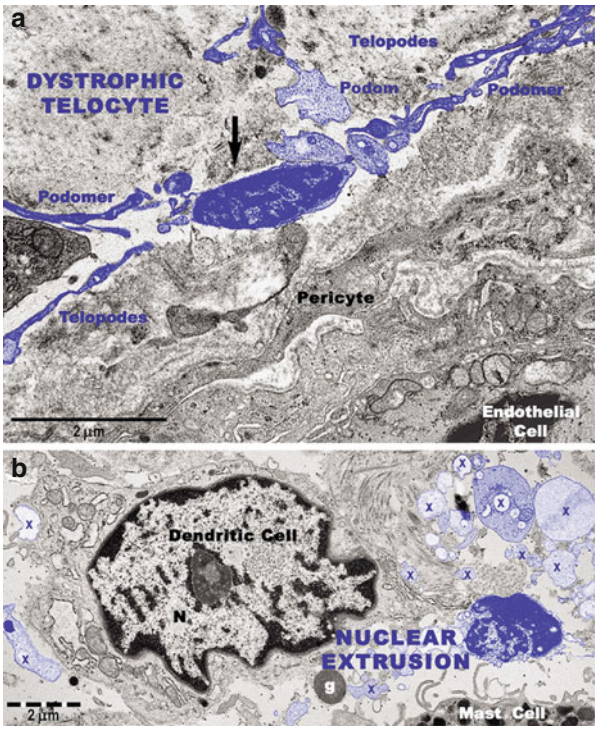

Fig. 4.10 Transmission electron microscopy images show degenerative changes in telocytes (digitally colored in *blue*) from psoriasis skin. (**a**) A telocyte with shriveled nucleus and detached telopodes. The *arrow* indicates dissolution of the cellular membrane and the cytoplasmic content surrounding the nucleus. (**b**) An extruded nucleus and cytoplasmic fragments (*X*) of a telocyte are visible in the vicinity of a dendritic cell. *g* granule (of a mast cell) (Reproduced with permission from Manole et al. [58])

morphology appears comparable in the dermis of uninvolved and treated skin but deeply decreases in the lesional psoriatic papillary dermis. Moreover, the few remaining telocytes exhibit degenerative ultrastructural features. Indeed, in psoriatic lesions, TEM revealed the presence of telocytes with apoptotic nuclei, dystrophic telocytes with fragmented telopodes, and even telocytes with nuclear extrusions, membrane disintegration, and cytoplasmic fragmentation (Fig. 4.10) [58]. Extruded nuclei or apoptotic telocytes were often observed to have close contacts with dendritic cells in the dermis of psoriatic skin. Profound changes in the phenotype of vascular smooth muscle cells (i.e., cells exhibiting a synthetic phenotype with decreased actin filaments and increased rough endoplasmic reticulum) in small blood vessels that lost the protective envelope formed by telocytes were also found. Therefore, it has been suggested that the loss of perivascular telocytes might have important implications in the characteristic vascular pathology of psoriasis [58]. Collectively, the authors proposed that the reduction in telocytes and their interstitial network may significantly influence psoriatic lesion initiation and/or progression, impairing long-distance heterocellular communication. Accordingly, in psoriasis telocytes could be considered as new cellular targets for forthcoming therapies. Besides psoriasis vulgaris, it will be of major importance to clarify the possible involvement of telocytes in other forms of psoriasis, such as pustular and erythrodermic psoriasis.

4.7 Concluding Remarks

Increasing evidence indicates that telocytes are a peculiar interstitial cell type implicated in tissue homeostasis and development [13, 14], as well as in the pathophysiology of several disorders [14, 22, 23, 27–29, 58–66]. In particular, severe damage and a broad reduction of telocytes have been reported during fibrotic remodeling of multiple organs in various diseases, including SSc, CD, UC, and liver fibrosis, as well as in chronic inflammatory lesions like those of pSS and psoriatic skin [14, 22, 23, 27–29, 58, 59]. Although several hypotheses have been proposed, the pathogenetic mechanisms underlying the loss of telocytes and their functional consequences in those disorders need to be further investigated.

Owing to their close relationship with stem cells and/or their capacity to guide or nurse putative progenitor cells in tissue-resident stem cell niches, telocytes are also supposed to contribute to tissue repair/regeneration [14, 55]. Indeed, telocytes are universally considered as "connecting cells" mostly oriented to intercellular signaling within the stromal compartment of almost every organ. Thus, a deeper understanding of how telocytes communicate with neighboring cells and take effect in signaling pathway during tissue repair/regeneration appears crucial to identify novel therapeutic strategies for the aforementioned and, possibly, other disorders. Interestingly, telocytes were found to be reduced during experimental myocardial infarction, particularly in fibrotic areas, and transplantation of cardiac telocytes could decrease the infarction size and improve postinfarcted cardiac function through the reconstruction of the telocyte network and the reduction of cardiac fibrosis [65, 66]. On this basis, in the near future, telocyte transplantation might represent a promising therapeutic opportunity to control the evolution of chronic inflammatory and fibrotic diseases. Notably, there is evidence to support that telocytes could help in preventing abnormal activation of immune cells and fibroblasts, as well as in attenuating the altered ECM organization during the fibrotic process [55]. By targeting telocytes alone or in tandem with stem cells, we might be able to promote regeneration and prevent the evolution to irreversible tissue damage. Finally, besides exogenous transplantation, exploring pharmacological or non-pharmacological methods to enhance the growth and/or survival of telocytes could be an additional therapeutic strategy for many disorders.

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