Autophagy, tissue repair, and fibrosis: a delicate balance

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Abstract

Tissue repair and fibrosis, an abnormal form of repair, occur in most human organs in response to injury or inflammation. Fibroblasts play a major role in the normal repair process by differentiating into myofibroblasts that synthesize extracellular matrix (ECM) components and favor tissue remodeling to reestablish normal function and integrity. However, their persistent accumulation at the site of injury is a hallmark of fibrosis. Autophagy is a catabolic process that occurs in eukaryotic cells as a stress response to allow cell survival and maintenance of cellular homeostasis by degrading and recycling intracellular components. Recent advances identify autophagy as an important regulator of myofibroblast differentiation, tissue remodeling, and fibrogenesis. In this mini-review, we provide an overview of the interactions between autophagy, ECM, and fibrosis, and emphasize the molecular mechanisms involved in myofibroblast differentiation. We also describe the emerging concept of secretory autophagy as a new avenue for intercellular communication at the site of tissue injury and repair.

Introduction

Tissue repair refers to the restoration of tissue architecture and function after injury. The capacity to repair damaged tissues in response to injury or inflammation is essential for the survival of eukaryotes. Tissue repair occurs through two types of reactions: regeneration of injured tissue and formation of scars that result from the excessive deposition of connective tissue. Repair leads to complete tissue restoration without any lingering trace of the initial aggression and of the inflammatory response that followed. This can be observed after limited destructive aggressions in tissues capable of cell regeneration. In contrast, scar formation occurs when the damaged tissue cannot regenerate (e.g. neurons or myocardial muscle cells) or when tissue destruction is extensive or prolonged [1]. The repair process involves numerous growth factors and complex interactions between cells and the extracellular matrix. Fibroblasts, alongside with endothelial cells [2], play a major role in the synthesis of the extracellular matrix, which in turn helps maintain tissue integrity. Fibroblasts possess contractile properties that allow them to migrate and invade the wound site [3]. Cytokines present at the site of injury along with local modification in the extracellular matrix induce the differentiation of fibroblasts into myofibroblasts. Myofibroblast can originate from mesenchymal cells, progenitor cells, smooth muscle cells, and hepatic stellate cells. They can also originate from the epithelial to mesenchymal transdifferentiation (EMT) of an epithelial cell. As fibroblasts is the best studied and most common source of myofibroblasts, myofibroblast differentiation described in this review will refer to that of fibroblasts. Differentiated myofibroblasts secrete extracellular matrix (ECM) components such as collagen, elastin, fibronectin, and proteoglycans like perlecan and decorin [4] and acquire a contractile phenotype through enhanced expression of alpha-smooth muscle actin and formation of stress fibers. Myofibroblasts are essential effectors of normal tissue repair by promoting tissue remodeling and wound closure. During fibrosis, a scarring type of repair, persistent myofibroblast accumulation at sites of injury fuels inappropriate ECM deposition and tissue contraction, culminating in organ and/or tissue

dysfunctions [5]. Thus, the presence of myofibroblasts at the injury site is a normal finding, while their unabated accumulation is a hallmark of scarring and fibrosis.

Autophagy is a stress response of eukaryotic cells that allows the degradation and recycling of intracellular components. It behaves as an adaptive response allowing cell survival in the absence of nutrients. It also helps maintain cellular homeostasis allowing the recycling of proteins and non-functional organelles. Various conditions such as ischemia, fasting, birth, and exercise induce autophagy [6]. Three main types of autophagy have been described: macroautophagy, microautophagy, and chaperone-mediated autophagy. Macroautophagy is characterized by the sequestration of part of the cytoplasm, including organelles, in a vacuole formed by a double membrane called autophagosome. The latter fuses with the lysosome to form an autolysosome where its content is degraded. Autophagy related (ATG) proteins are key players in the regulation of autophagy. More than 30 ATG proteins have been identified so far and are involved in the biogenesis, elongation, and closure of the autophagosome membrane. Among these ATG proteins, ATG7 allows the activation and ubiquitination of the Microtubule Associated Protein 1 Light Chain 3 Beta (LC3) followed by conjugation to ATG3. This will then allow the lipidation of LC3 and its recruitment to the autophagosome membrane [7]. ATG7 may also allow the formation of the ATG12-ATG5-ATG16 complex which determines the LC3 lipidation site [8]. During chaperone-mediated autophagy, proteins carrying a specific sequence are recognized by the chaperone Hsp70. This complex associates with the lysosome membrane protein Lamp2 inducing its oligomerization. The targeted protein is then translocated in the lysosome using Hsp70 and Lamp2 where it is degraded. Microautophagy is characterized by the sequestration of part of the cytoplasm by the lysosome membrane directly [9]. All three types of autophagy converge towards the lysosome which allows the degradation of proteins by lysosomal hydrolases. Emerging data suggest that autophagosome components can also be released instead of being degraded, suggesting that autophagy can sometimes behave as a non-classical

secretion pathway [10-12]. The autophagy process described in this review refers to macroautophagy (Figure 1A). In recent years, findings of dysregulated autophagy in association with development of fibrosis have fueled an increasing interest on the molecular cross-talk between both pathways. In this review, we summarize recent advances in our understanding of the molecular pathways controlling interactions between the ECM, autophagy, myofibroblast differentiation and fibrosis.

Molecular crosstalk between autophagy and myofibroblast differentiation

Both activation and inhibition of autophagy have been associated with myofibroblast differentiation in various stress conditions highlighting the potentially diverse roles of autophagy in the different phases of stress response and tissue repair (Tables 1 and 2). In a rat skin model, spatiotemporal analysis of the autophagic marker LC3 during the wound healing process showed that functional changes in fibroblasts correlate with alteration of the autolysosomal degradation system [13]. Multiple studies in different organs have shown a positive correlation between activated autophagy and myofibroblast differentiation during tissue regenerative processes. Plating adult rat cardiac fibroblasts on a non-compressible substrate was associated with the mechanical induction of autophagy and induction of a myofibroblast phenotype. Autophagy induction correlated with the inhibition of the p38 mitogen-activated protein kinases (MAPK) signaling pathway [14]. In a laminectomy model in rats, effective tissue regeneration and control of epidural scar hyperplasia were associated with autophagy. In this study, laminectomy sites presenting scattered epidural scar tissue featured an increase in autophagy-related protein expression [15]. Studies of fibroblast activation dynamics in human fibroblasts derived from oral mucosa or gingiva from healthy tissue demonstrated a causal role for autophagy in myofibroblast differentiation. In this setting, oral mucosa fibroblasts displayed higher a-SMA and collagen deposition after surgical incision along with autophagy activation. In contrast, gingiva fibroblasts lacking the capacity to mount an autophagic response failed

to differentiate into myofibroblasts [16]. These studies highlight the need for fine control of autophagy in specific tissue fibroblasts to allow normal tissue repair without fibrosis and scar formation.

Modulation of autophagy has also been associated with the development of fibrosis and myofibroblast differentiation of hepatic stellate cells (HSC) (reviewed in Ref. [17, 18]). Autophagy-dependent activation of HSC into fibrogenic myofibroblasts is considered the central driver of hepatic fibrosis. Autophagy induces myofibroblast differentiation of HSC by generating fatty acids from the cleavage of retinyl esters stored in lipid droplets [19]. Blocking autophagy using pharmacological inhibitors and knockdown of ATG7 attenuated fibrogenic activity [20]. Autophagy counteracts fibrogenesis in the liver by favoring cellular homeostasis in hepatocytes, macrophages, and liver endothelial cells [21]. These results highlight the need to take into consideration cell type-specific autophagy programs and different kinetics of autophagy activation that may contribute either to normal tissue repair or to perpetuation of myofibroblast differentiation and fibrosis.

Autophagy also plays a pivotal role in fibrogenesis associated with inflammation where its inhibition suppressed myofibroblast phenoconversion in tlr4-activated cardiac fibroblasts [22]. Autophagy is also essential for the activation of renal interstitial fibroblasts in hyperuricemia-induced kidney injury. In this model, autophagy inhibition decreases renal accumulation of ECM proteins and fibroblast activation [23]. We reported that human embryonic lung fibroblasts with prolonged autophagy induced by serum starvation produce increased levels of connective tissue growth factor (CTGF) which in turn induces myofibroblast differentiation. Interestingly, myofibroblast differentiation was induced by TGFB-independent pathways, as demonstrated by the absence of SMAD signaling and failure of TGFB neutralizing antibodies to prevent autophagy-induced myofibroblast differentiation [24]. CTGF is a matricellular protein involved in extracellular matrix remodeling [25] and is upregulated in various chronic fibrotic disorders [26, 27]. It functions as a downstream effector of TGFB's fibrogenic actions [28], but can also induce myofibroblast

differentiation and fibrosis through TGFB-independent pathways [29, 30]. TGFB also regulates autophagy and myofibroblast differentiation, although the various levels of regulation and cross-talk between the two pathways remain ill-defined. Some studies have demonstrated in different fibroblast systems that TGFB promotes autophagy, while others suggested the opposite. TGFB favors kidney fibroblast differentiation by inducing autophagy through PKCa and S473-Akt activation, two important downstream targets of the mammalian target of rapamycin complex 2 (MTORC2) [31, 32]. Another study also showed that autophagy induction is a requisite for TGFB-induced fibrogenesis in primary human atrial fibroblasts by targeting ATG5 and 7 [33]. Interestingly, many studies suggesting that autophagy plays a protective role in myofibroblast differentiation used rapamycin as an autophagy inducer. They concluded that the induction of autophagy inhibits myofibroblast activation and alleviates TGFB-induced fibrogenesis. It should be noted that these studies did not investigate the effect of rapamycin per se on the respective activity of mTORC1 and mTORC2 [34-37]. Recent and mounting evidence suggests a key role for mTORC2 in the regulation of myofibroblast differentiation [24, 38-40]. TGFB has also been shown to reduce autophagy by repressing the expression of several ATGs, including ATG5 and 7, and p62 in normal lung fibroblasts [41]. Inhibition of autophagy with knockdown of LC3B and ATG5 also increased the expression of myofibroblast markers in fibroblasts extracted from samples of patients with idiopathic pulmonary fibrosis (IPF) [42]. IPF fibroblasts naturally displayed a persistent decrease in autophagy which was associated with a propensity towards fibrogenesis [43].

These conflicting results on the role of autophagy in fibrogenesis could be explained by a number of factors. The duration of autophagy and the environmental conditions may explain why the knock-down of ATG7 in normal lung fibroblasts under normal conditions promoted the expression of aSMA and Col1 [43], while inhibiting myofibroblast differentiation during persistent serum starvation [24, 38]. Conflicting studies using rapamycin also suggest sophisticated interactions between

autophagy and MTORC1/MTORC2 complexes. MTOR is a serine/threonine-protein kinase and a member of the phosphoinositide 3-kinase (PI3K)-related kinase family. It is part of two different protein complexes: MTORC1 and MTORC2. MTORC1 complex is composed of MTOR, the regulatory-associated protein of mammalian target of rapamycin (RAPTOR), proline-rich Akt substrate 40kDa (PRAS40), mammalian lethal with sec-13 (mLST8/GβL), DEP domain-containing mTOR-interacting protein (DEPTOR), Tti1 and Tel2. MTORC2 complex contains, in addition to MTOR, mLST8, DEPTOR, Tti1, and Tel2 which are common to both complexes, rapamycin-insensitive companion of mTOR (RICTOR), mammalian stress-activated map kinase-interacting protein 1 (mSin1), and protein observed with rictor 1 and 2 (protor1/2) [44]. MTORC1 was originally described as the target of rapamycin, while the MTORC2 complex was defined as being insensitive to rapamycin (hence the name RICTOR for rapamycin-insensitive companion of mammalian target of *rapamycin*) [45]. However, subsequent studies demonstrated that the MTORC2 complex can be inhibited by rapamycin, under certain conditions, and in certain cell types. Prolonged exposure to rapamycin prevents the formation of new MTORC2 complexes, thereby inhibiting its activity [46]. MTORC1 is mostly known to regulate mRNA translation and cell proliferation via the phosphorylation of p70 S6 kinase 1 (S6K1) and eukaryotic translation initiation factor 4E binding protein 1 (4E-BP1). MTORC2 activation regulates cell survival and reorganization of the actin cytoskeleton via the phosphorylation of serum and glucocorticoid-induced protein kinase 1 (SGK1), protein kinase C-α (PKCa), and Akt at Ser473 [44, 47]. However, it was demonstrated that MTORC2 is also involved in the expression of stress and hypoxia-induced proteins of potential importance in controlling cellular adaptation to external stress [48] (Figure 1B). Some studies showed that MTORC1 was the primary MTOR complex driving myofibroblast differentiation in IPF fibroblasts, consistent with a protective role for autophagy in these conditions [49, 50]. However, a growing body of evidence also points to a central role of MTORC2 in the modulation of myofibroblast activation (Table 3). It was demonstrated that the dual inhibition of MTORC1/2 disrupted TGFB-induced myofibroblast phenotype in lung

fibroblasts [39] and selective inhibition of MTORC2-Akt-S473 axis in pterygium fibroblasts impeded TGFB-induced myofibroblast differentiation [40]. Moreover, strong MTOR expression and aberrant activation of S473-Akt has been seen in IPF lung, suggesting a potential role for MTORC2 in this fibrotic disorder [51, 52]. It has also been shown that MTORC2 signaling can mediate ECM deposition in mesenchymal cells upstream of MTORC1 only in fibrotic conditions through an S473-Akt feedback loop [53]. How autophagy is related to MTORC2 activation remains unclear. Our group demonstrated a distinct pattern in MTORC2 signaling in starved fibroblasts, where Akt phosphorylation rapidly decreased upon starvation followed by spontaneous reactivation after 2 days. The inhibition of autophagy by silencing ATG7 abolished myofibroblast differentiation driven by MTORC2, thus identifying autophagy as an upstream regulator of MTORC2 [24, 38]. Positive regulation of MTORC2 by autophagy has also been described in colorectal cancer cells, where autophagy mediates receptor tyrosine kinase (RTK) phosphorylation via regulation of mTORC2 [54]. Our group identified reactive oxygen species (ROS) as important mediators of autophagy-induced MTORC2 activation and myofibroblast differentiation [38]. Starvation by glutamine-depletion was also shown to induce ROS-dependent MTORC2 activation in non-small cell lung cancer cells [55]. Aside from being activated by ROS, MTORC2 is also involved in ROS metabolism by regulating the redox state [56]. The levels of crosstalk between autophagy, ROS production, and MTORC2 activation are complex and incompletely defined but dependent on cellular origin and environmental clues and stress that contribute to, and regulate, myofibroblast differentiation, ECM deposition, and fibrosis.

Impact of the ECM in the regulation of autophagy and fibrogenesis

Excessive ECM deposition is a hallmark of fibrotic disorders. In the last decade, there has been a growing focus on the impact of ECM components on autophagy and downstream myofibroblast differentiation. A variety of matrix constituents, such as collagen, decorin, endostatin, and perlecan, have emerged as regulators of autophagy. These matrix-derived products modulate both autophagy and

fibrogenesis. While other articles have reviewed ECM-driven autophagy in different cell types, [57, 58], here we present the impact of ECM constituents on the regulation of autophagy in fibroblasts and their role in myofibroblast differentiation.

Decorin, a small leucine-rich proteoglycan, can evoke autophagy in fibroblasts in a way similar to what is found in endothelial cells. Decorin induces autophagy through interactions with the VEGFR2 receptor and concomitant activation of AMP-activated protein kinase leading to induction of paternally expressed gene 3 (PEG3) expression [59]. Interestingly, autophagy induced by serum starvation in mouse embryonic fibroblasts can also increase decorin expression. This work suggests that decorin itself can be modulated by autophagic stimuli and initiate a potential positive feedback loop [60]. Whether decorin-induced autophagy modulates myofibroblast differentiation and fibrogenesis has yet to be determined, but decorin is usually associated with anti-fibrotic properties in a number of animal models [61, 62].

Endostatin, resulting from the C-terminal cleavage of collagen XVIII-a1 [63], inhibits TGFB-induced myofibroblast differentiation by a PDGFR/ERK-dependent signaling pathway [64]. However, the effect of endostatin on fibroblast autophagy remains ill-defined. Endostatin can induce autophagy of endothelial cells through interactions with a5b1 integrins [65]. Whether this mechanism can also be evoked in fibroblasts remains to be evaluated.

Collagen VI (ColVI), a major structural ECM component, plays a crucial role in tissue homeostasis and function [66]. Recently, a study showed that extracellular collagen VI (ColVI) modulates autophagy in murine embryonic fibroblasts. Lack of ColVI leads to a dysfunctional autophagic flux with the accumulation of autophagosomes; adhesion of Col6a1−/− fibroblasts onto ColVI normalized the autophagic flux [67]. Coincidentally, it was demonstrated that Col VI orchestrates a profibrotic phenotype in human myofibroblasts by increasing their secretion of chemokines and enhancing their migration and contractility [68]. Denatured collagen I (ColI) also

regulates autophagy and myofibroblast differentiation. Collagen denaturation occurs as a consequence of mechanical stress and damage, enzymatic degradation, or matrix remodeling. Different studies demonstrated that denatured ColI induces autophagy in fibroblasts [69], but also promotes differentiation of human fibroblasts [70]. Whether myofibroblast differentiation triggered by ColVI and denatured Coll occurs downstream of autophagy activation remains to be determined. It is plausible that collagen reorganization and stress in damaged tissues could initiate a positive feedback loop between myofibroblasts and their environment to promote wound healing or exacerbate fibrosis [71].

Perlecan is a large, multi-domain heparan sulfate proteoglycan, embedded within the vascular basement membrane [72]. The functional properties of perlecan in its native form and of perlecan fragments such as endorepellin and LG3 have been shown to differ on a number of aspects (angiogenesis, apoptosis, migration, etc). The regulation of autophagy by perlecan is no exception. In its native form, perlecan acts as an autophagy inhibitor. Using conditional Hspg2 −/− mice, a study showed that muscle tissue lacking perlecan display an increased number of autophagosomes in association with inhibition of the MTOR pathway [73]. To date, there is no evidence that endorepellin or the C-terminal LG3 domain can regulate autophagy in fibroblasts. However, endorepellin induces autophagy in a way similar to decorin through VEGFR2 and PEG3 signaling in endothelial cells [74]. Our group also demonstrated that soluble LG3 released by apoptotic endothelial cells binds b1-integrins on fibroblasts and activates an AKT-dependent pathway leading to myofibroblast differentiation [75, 76]. We also demonstrated that LG3 injection fostered a fibroproliferative response with the accumulation of aSMA-positive cells in an allograft vascular rejection model [77].

Periostin, a matricellular protein that binds both ECM components and cell surface receptors, has been increasingly associated with fibrogenesis in various organs both in the context of development and in disease states. Periostin was found to promote myofibroblast differentiation of lung fibrocytes

[78] and fibroblasts in the skin [79] and heart [80]. For instance, the absence of periostin impairs a-SMA expression and alters wound-closure kinetics; exogenous periostin on Postn−/− dermal fibroblasts restores a myofibroblast phenotype [79]. Few studies have looked at the impact of periostin on autophagy. Recently, it was shown that periostin impairs autophagy in tubular epithelial cells therefore promoting renal fibrosis and inflammation in nephrectomized rats. Inhibition of periostin using shRNA reduces renal fibrosis and inflammation along with the restoration of the autophagic flux [81]. Whether periostin can also regulate autophagy in fibroblasts to promote fibrosis remains to be defined.

Osteopontin is a multifunctional extracellular matrix protein expressed in the bone matrix and in various organs. It can act both as a structural molecule and as a cytokine. Several studies using OPN-/- fibroblasts highlighted the essential role of osteopontin in myofibroblast differentiation triggered by TGF-B [82-84]. A recent study suggests that osteopontin-induced myofibroblast differentiation is linked to inhibition of autophagy [85].

Fibronectin, as a core component of ECM, regulates cell activities mainly through interaction will cell surface integrin receptors. In fibroblasts, binding of fibronectin with integrins mediates fibroblast survival [86, 87]; whereas lack of binding leads to autophagy [88]. Studies suggest that fibronectin could inhibit autophagy through the activation of AKT/MTOR signaling pathway [89, 90]. However, its role in fibrosis may differ dependent on the organ and its splice variant. It was demonstrated that fibronectin promotes fibrosis and myofibroblast differentiation whereas the inhibition of its polymerization attenuates myofibroblast differentiation in cardiac fibroblasts and fibrosis secondary to myocardial ischemia in mice [91]. The alternatively spliced domain A (EDA)-fibronectin also induces myofibroblast differentiation of lung fibroblasts through a4b7-integrin-binding [92]. In contrast, fibronectin attenuates stellate cells-induced liver fibrosis by controlling the availability of active TGF-B [93].

Collectively, these various pieces of work demonstrate a pivotal role for ECM components in the regulation of stress responses such as autophagy and myofibroblast differentiation, which could, if not properly balanced, favor the development of fibrosis (Figure 2 and Table 4). It also highlights the importance of integrins as key communicators between the extracellular environment, autophagy, and myofibroblast differentiation. Further studies are needed to better characterize the complex levels of molecular cross-talk between ECM components, integrin-dependent signaling, and downstream regulation of autophagy and tissue repair.

Senescence interplays with autophagy to regulate myofibroblast differentiation

Cellular senescence refers to a stable arrest of the cell cycle. Several stimuli are known to induce senescence, including activated oncogenes, telomere shortening, and oxidative stress [94]. DNA repair pathways converging towards the activation of the TP53-CDKN1A/p21WAF1 and CDKN2A/p16INK4a-RB pathways are important inducers of senescence-related cell cycle arrest. Senescent cells also present an increase in senescence-associated GLB1/beta-galactosidase (SA-GLB1/β-gal) activity in lysosomes [95]. Mounting evidence suggests that senescence contributes to tissue repair while dysregulated senescence favors scarring or fibrogenesis [3, 96-99]. Fibrosis has been linked to both enhanced [100] and decreased [101, 102] cellular senescence. Genetic invalidation of the central senescence gene TP53 increased liver fibrosis in a murine model of liver injury. Mice with deletion of the matricellular protein CCN1 in hepatocytes developed fibrosis concomitantly with a deficit in cellular senescence [101]. CCN1 knockout mice presented decreased p16- and p53-pathway activation after injury and enhanced fibrosis in a cutaneous wound healing model. It was showed that CCN1 induced senescence in skin fibroblast by an a6b1 integrin-mediated ROS generation mechanism leading to a decreased expression of type I collagen [103]. Similarly, in a COPD-like *in vitro* model, oxidative stress induced by the herbicide paraquat triggered senescence in parenchymal lung fibroblasts. Consequently, it resulted in a significant decrease in the expression of several ECM

296 components such as type I collagen, elastin, and fibronectin, along with the inhibition of the
297 myofibroblast markers, aSMA. [104]. In contrast, studies using senescent fibroblasts derived from IPF
298 patients showed increased levels of ECM genes, notably, type I collagen in association with
299 overexpression of aSMA [105-107]. These conflicting outcomes likely suggest that the duration of the
300 senescence process, the cell types involved and the downstream pathways triggered by senescence
301 result from complex molecular interactions that may be either beneficial or harmful to normal tissue
302 repair.

303 Senescence and autophagy have generally been considered two distinct cellular stress
304 responses, but mounting evidence suggests that the two are functionally interconnected [108, 109].
305 Autophagy impairment, by depletion of ATG7 and 12, and lysosomal-associated membrane protein 2
306 (Lamp2) in human fibroblasts, induced premature senescence in a TP53-dependent and ROS-
307 dependent manner [110]. Recent work also suggested an inverse relationship between senescence and
308 autophagy. Autophagy inhibition was associated with the upregulation of senescence in TGFB-induced
309 myofibroblast differentiation of IPF-derived human fibroblasts [111]. However, the actual conclusion
310 derived from this work is that senescence and autophagy are *associated*, but not necessarily opposed.
311 Indeed, autophagy was assessed in this study using only the LC3 marker. It is now appreciated that the
312 amount of lipidated LC3 at a given time point is not a reliable marker of autophagic flux [112]. In other
313 studies, autophagy and senescence were found to be interconnected; inhibition of autophagy with
314 ATG5 or 7 silencings resulted in delayed oncogene-induced senescence in human lung fibroblasts
315 [113]. Fibroblasts overexpressing ATG16L1 to activate autophagy showed induction of senescence
316 markers such as p21 and p19 coupled with b-gal activity [114]. Likewise, ROS-induced autophagy
317 controlled senescence through increased expression of p21 by a p38 MAPK-dependent pathway in
318 human embryonic fibroblasts. Interestingly, the MTORC2 downstream target, S473-AKT was not
319 inhibited during the process [115]. There is also evidence that modulation of senescence can influence

autophagy and fibroblast phenotype; induction of cellular senescence by hyperoxia in human fetal lung fibroblasts resulted in impaired autophagy, shown by a decrease in LC3-II and beclin-1 protein expression, and increased ECM deposition, as collagen IV [116].

Recently, our group demonstrated that sustained autophagy in serum-starved fibroblasts enhances ROS production leading to enhanced MTORC2 activity and concurrent activation of senescence and myofibroblast differentiation. However, at the cellular level, senescence and myofibroblast differentiation were mutually exclusive; myofibroblasts lacking senescent markers and vice-versa. In the long term, however, senescence triggered a positive activation feedback loop sustaining MTORC2 activation while at the same time preventing myofibroblast differentiation [38]. How MTORC2 activation induced by autophagy-dependent-ROS controls the fate of fibroblasts toward either senescence or myofibroblast differentiation remains to be determined. However, this work highlighted the existence of molecular crosstalk between autophagy, senescence, and myofibroblast differentiation and helped reconcile studies where both activation and inhibition of autophagy is associated with myofibroblast differentiation (Figure 3).

Autophagy, non-conventional secretion, and ECM proteolysis

Emerging data suggest that autophagy can behave as an unconventional secretion pathway. Traditionally, the autophagic machinery leads to the degradation of the autophagosome content after its fusion with lysosomes. Unlike classical autophagy, secretory autophagy leads to the release of autophagosome constituents instead of their degradation and controls the secretion of proteins that lack peptide signals.

The ECM, through its functions and integrity, is vital for tissue and organ homeostasis as well as for appropriate tissue repair. ECM proteolysis is orchestrated by multiple remodeling enzymes, of which the matrix metalloproteinases (MMP) and cathepsins (CTS) are best characterized. A number

of studies have identified autophagy as an important player in the secretion of these enzymes. Autophagy was found to play a crucial role in ECM proteolysis and oncogenic invasion. Autophagy inhibition by ATG7 or ATG12 depletion reduces MMP2 secretion in conditioned media from MCF10A cells impairing their RAS-driven invasion [117]. It was also shown that autophagic flux regulates the secretion of CTS in macrophages where knockdown of ATG genes and 3-MA treatment decreased CTS secretion [118, 119]. Whether the secretion of these proteases by autophagy also plays a role in tissue repair and fibrosis remains to be determined.

Secretory autophagy has also been implicated in the secretion of extracellular vesicles (EVs) [120, 121], membrane-bound vesicles secreted by most cells under physiological or pathological conditions. EVs vary widely in size from 50 nm to 5 μm and have a wide range of protein, nucleic acid, and lipid content [122]. EVs generated by the budding of the plasma membrane of a live cell are referred to as microvesicles or ectosomes. Invagination of the inner leaflet of multivesicular bodies (MVB) followed by their fusion with the cell membrane leads to the release of exosomes [123]. Studies have reported that autophagy can promote the secretion of both microvesicles and exosomes [124, 125]. Secretory autophagy occurs often in the context of inflammation and tissue remodeling, hinting at a potential role in tissue repair and fibrogenesis [126]. For instance, tribbles pseudokinase 3 (TRIB3), a stress protein, redirected the autophagic machinery toward a secretory pathway and stimulated exosome secretion from hepatocytes. Uptake of these EVs by hepatic stellate cells resulted in the accumulation of fibrosis-promoting factors, secretion of aSMA, and deposition of ColI thereby promoting the development of liver fibrosis [125]. Furthermore, we demonstrated that nutrient-deprived apoptotic endothelial cells can release extracellular vesicles containing autophagic components [10, 11, 127]. In recent years, our group also identified a novel type of EVs, apoptotic exosome-like vesicles (ApoExo), secreted by apoptotic endothelial cells downstream of caspase-3 activation. ApoExo differ from classical exosomes by their content and biological functions [128-131].

We showed that soluble LG3 is released in ApoExo by apoptotic endothelial cells and that autophagy was crucial in perlecan cleavage and loading of the LG3 fragment within ApoExo [12](Unpublished results). Whether LG3 within ApoExo is responsible for neointimal thickening observed in a murine model of transplant vasculopathy remains to be determined. However, we showed previously that increasing circulating LG3 levels in this model by intravenous injections of recombinant LG3 hastens neointima formation and accumulation of aSMA positive cells in a similar way to what was observed with the injection of ApoExo [131, 132]. These emerging data unveil a potential new role for autophagy in degradation and secretion of ECM components of importance in fibroproliferative responses.

Conclusion

Fibrosis is a chronic and progressive alteration in tissue architecture and function due to excessive accumulation of ECM. Autophagy is increasingly considered an important regulator of tissue remodeling during normal tissue repair and fibrosis, although its impact and specific role may differ dependent on the organ and duration of the triggering insult. Cellular senescence, another means of responding to stress, has also been linked to myofibroblast differentiation, tissue repair, and fibrosis. Autophagy and senescence both regulate myofibroblast differentiation and mounting evidence identifies MTORC2 as a converging signaling pathway to both autophagy and senescence of importance in determining the fate of fibroblasts toward pro- or anti-fibrotic phenotypes. ECM components also play an important role in the regulation of various stress responses including autophagy and senescence. Considering the complex levels of crosstalk between ECM components, autophagy and senescence, it will be crucial to gain further insights into the molecular effectors of cross-talk during normal tissue repair and abnormal scarring processes. For instance, how MTORC2 targets mutually exclusive signaling pathways leading to either senescence or myofibroblast differentiation remains to be determined. Therefore, characterizing the detailed molecular interplay between autophagy and senescence during normal repair and fibrosis should help provide a framework

of druggable targets of potential benefits in preventing maladaptive tissue repair and fibrosis. Finally, the emerging concept of autophagy contributing to the release of remodeling enzymes and EVs containing autophagosome and lysosome constituents opens new avenues for assessing the importance of ECM degradation and intercellular communication at sites of tissue injury and repair.

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Disclosure

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Figure 1. Autophagy and MTOR signaling pathways. (**A**) Mechanistic presentation of the different types of autophagy. (1) Macroautophagy, (2) Chaperone-mediated autophagy and (3) Microautophagy. (**B**) Molecular depiction of the two distinct MTOR protein complexes MTORC1 and MTORC2 and their respective downstream targets.

Figure 2. Impact of extracellular matrix components on autophagy and myofibroblast differentiation. Multiple matrix constituents modulate both autophagy and myofibroblast differentiation through different signaling pathways. * observed in other than fibroblast cell type

Figure 3. MTORC2 activation determines the fate of cells either toward senescence or myofibroblast differentiation. Stress signal induces autophagy. Sustained autophagy fosters MTORC2 activation in fibroblasts. MTORC2 activation determines the fate of fibroblasts either toward senescence or myofibroblast differentiation. Senescence concomitantly maintains MTORC2 activation while suppressing myofibroblast differentiation.

Table 1. Role of autophagy in fibrosis and myofibroblast differentiation

Cell source	Tissue	Myofibroblast differentiation	Markers	Signaling	Reference
Rat cardiac fibroblasts	Heart (atria, ventricles)	promotes	α-SMA, fibronectin	p38 MAPK inhibition	[14]
Mouse cardiac fibroblasts	Heart (atria, ventricles)	promotes	α-SMA	TLR4 signaling pathway	[22]
NRK-49F	Kidney	promotes	α-SMA, collagen I	-	[23]
Oral mucosa fibroblasts	Oral mucosa, Gingiva	promotes	α-SMA, collagen I	-	[16]
WI-38, NHLF, MEF	Lung, Embryo	promotes	α-SMA, collagen I, collagen III, stress fibers	TGFβ-independent MTORC2 activation	[24]
NRK-49F	Kidney	promotes	α-SMA, fibronectin, collagen	TGFβ-dependent MTORC2 activation	[31]
Human cardiac fibroblasts, MEF	Heart (atria)	promotes	fibronectin, collagen I	TGFβ-dependent SMAD activation	[33]
NIH-3T3, rat tenocytes	Tendons	impedes	α-SMA, collagen I, collagen III	-	[34]
Human subconjunctival fbroblasts	Conjunctiva	impedes	α-SMA, fibronectin, collagen I	-	[35]
MRC-5	Lung	impedes	α-SMA, fibronectin, collagen I	inhibition of PI3K/AKT/mTOR pathway	[36]
Human intestinal fibroblasts	Intestine (colon)	impedes	collagen I, collagen III	-	[37]
WI-38, NHLF	Lung	promotes impedes	α-SMA	MTORC2 activation senescence induction	[38]
NHLF	Lung	impedes	α-SMA, collagen I	-	[41]
NHLF	Lung	impedes	α-SMA, collagen I	-	[42]

NHLF : normal human lung fibroblasts, WI-38: WI-38 normal human fibroblasts, MEF: mouse embryonic fibroblasts, MRC-5: human fetal lung fibroblast cell line, NIH-3T3: embryo murine fibroblasts, NRK-49F: Rat renal interstitial fibroblasts

Table 2. Models used to manipule autophagy in fibroblasts and fibrosis

Cell source	Model	Autophagy modulation	Impact on myofibroblast differentiation	Reference
Rat cardiac fibroblasts	Pharmacological (Baf-A1, CQ)	inhibition	impedes	[14]
Mouse cardiac fibroblasts	Pharmacological (3-MA)	inhibition	impedes	[22]
NRK-49F	Pharmacological (3-MA)	inhibition	impedes	[23]
Oral mucosa fibroblasts	Pharmacological (CQ)	inhibition	impedes	[16]
WI-38, NHLF, MEF	Serum starvation Pharmacological (Baf-A1, CQ, 3-MA, LY) Genetic (siATG7)	activation inhibition inhibition	promotes impedes impedes	[24]
NRK-49F	Pharmacological (CQ, 3-MA, Go) Genetic (siPKCα)	inhibition inhibition	impedes impedes	[31]
Human cardiac fibroblasts, MEF	Pharmacological (Baf-A1, 3-MA) Genetic (shATG7, ATG5 KO) Pharmacological (Rapa)	inhibition inhibition activation	impedes impedes promotes	[33]
NIH-3T3, rat tenocytes	Pharmacological (Rapa) Pharmacological (3-MA) Genetic (siATG5)	activation inhibition inhibition	impedes promotes promotes	[34]
Human subconjunctival fbroblasts	Pharmacological (TRE, Rapa) Pharmacological (CQ)	activation inhibition	impedes promotes	[35]
MRC-5	Pharmacological (ISL, Rapa) Pharmacological (3-MA, LY)	activation inhibition	impedes promotes	[36]
Human intestinal fibroblasts	Pharmacological (Rapa) Pharmacological (Baf-A1, 3-MA)	activation inhibition	impedes promotes	[37]
WI-38, NHLF	Serum starvation Pharmacological (3-MA, LY) Genetic (siATG7)	activation inhibition inhibition	promotes impedes impedes	[38]
NHLF	Pharmacological (CQ) Pharmacological (RSV, Torin1, Tat-beclin 1) Serum starvation	inhibition activation activation	promotes impedes impedes	[41]

Baf-A1: bafilomycin-A1, CQ: chlroquine, 3-MA: 3-methyladenine, LY: LY294002, Go: Go6976, Rapa: rapamycin, TRE: trehalose, ISL: isoliquiritigenin, RSV: resveratrol

Table 3. Role of MTOR pathway in fibrosis and myofibroblast differentiation

Cell source	Tissue	MTOR pathway	Myofibroblast differentiation	Markers	Reference
WI-38, NHLF, MEF	lung, embryo	MTORC2 (pS473-Akt)	promotes	α-SMA, collagen I, collagen III, stress fibers	[24]
NRK-49F	kidney	MTORC2 (PKCα, pS473-Akt)	promotes	α-SMA, fibronectin, collagen	[31]
NRK-49F	kidney	MTORC2 (PKCα, pS473-Akt)	promotes	α-SMA, fibronectin, collagen I	[32]
WI-38, NHLF	lung	MTORC2 (pS473-Akt)	promotes impedes	α-SMA	[38]
NHLF	lung	MTORC1 (S6K1, 4E-BP1), MTORC2 (pS473-Akt)	promotes	α-SMA, fibronectin, collagen I, stress fibers	[39]
human pterygium fibroblasts	conjunctiva	MTORC2 (pS473-Akt)	promotes	α-SMA, fibronectin, collagen I	[40]
NHLF	lung	MTORC1	promotes	α-SMA, collagen I	[49]
NHLF	lung	MTORC1 (4E-BP1)	promotes	α-SMA, collagen I	[50]
mesenchymal cells	lung	MTORC1 (S6K1, 4E-BP1), MTORC2 (pS473-Akt)	promotes	collagen I	[53]

Table 4. Impact of ECM constituents on the regulation of autophagy and myofibroblast differentiation in fibroblasts

ECM components	Autophagy modulation	Myofibroblast differentiation	Reference
Decorin	activation	-	[59]
	-	impedes	[61, 62]
Endostatin	-	impedes	[64]
	activation*	-	[65]
Collagen VI	activation	-	[67]
	-	promotes	[68]
Denatured collagen I	activation	-	[69]
	-	promotes	[70]
Perlecan (native)	inhibition*	-	[73]
Perlecan (endorepellin)	activation*	-	[74]
Perlecan (LG3)	-	promotes	[75-77]
Periostin	inhibition*	promotes	[81]
	-	promotes	[78-80]
Osteopontin	inhibition	promotes	[85]
	-	promotes	[82-84]
Fibronectin	inhibition	-	[89, 90]
	-	promotes	[91, 92]
	-	impedes	[93]

* not in fibroblast