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Review

Review

Autophagy and skin wound healing

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Abstract

Autophagy is a lysosome-dependent, self-renewal mechanism that can degrade and recycle cellular components in eukaryotic cells to maintain the stability of the intracellular environment and the cells ability to cope with unfavorable environments. Numerous studies suggest that autophagy participates in regulating various cellular functions and is closely associated with the onset and progression of various diseases. Wound healing is a complex, multistep biological process that involves multiple cell types. Refractory wounds, which include diabetic skin ulcers, can seriously endanger human health. Previous studies have confirmed that autophagy plays an essential role in various phases of wound healing. Specifically, in the inflammatory phase, autophagy has an anti-infection effect and it negatively regulates the inflammatory response, which prevents excessive inflammation from causing tissue damage. In the proliferative phase, local hypoxia in the wound can induce autophagy, which plays a role in anti-apoptosis and anti-oxidative stress and promotes cell survival. Autophagy of vascular endothelial cells promotes wound angiogenesis and that of keratinocytes promotes their differentiation, proliferation and migration, which is conducive to the completion of wound re-epithelialisation. In the remodeling phase, autophagy of fibroblasts affects the formation of hypertrophic scars. Additionally, a refractory diabetic wound may be associated with increased levels of autophagy, and the regulation of mesenchymal stem cell autophagy may improve its application to wound healing. Therefore, understanding the relationship between autophagy and skin wound healing and exploring the molecular mechanism of autophagy regulation may provide novel strategies for the clinical treatment of wound healing.

Key words: Autophagy, Wound healing, Diabetes, Mesenchymal stem cells, microRNA, Skin

Highlights

- Chronic wounds are caused by local tissue defects and necrosis and characterized by delayed wound healing, which seriously affects the quality of life of patients.
- The mechanisms and functions of autophagy in skin wound healing are intricate.
- Targeting autophagy may represent a novel strategy for the treatment of chronic refractory wounds.

Background

Skin wound healing is a complex and finely regulated biological process involving interactions between multiple cell types and mediators. Refractory wounds, which include diabetic skin ulcers, seriously endanger human health. Autophagy is a highly conserved eukaryotic cellular recycling process that plays a vital role in cell survival and maintenance. Dysregulated autophagy has implications in health and disease. An increasing number of studies show that autophagy is particularly essential for wound healing and repair; however, our current understanding of the systematic relationship between wound healing and autophagy is insufficient. In this review, we summarize the updated research progress on autophagy and skin wound healing and focus on its clinical relevance to offer a novel therapeutic strategy.

Review

Skin wound healing

Wound healing is a highly ordered biological process under normal physiological conditions. In general, this process comprises three independent yet overlapping phases: the hemostasis/inflammatory, proliferative and remodeling phases [1]. Wound formation is followed by the constriction of blood vessels and initiation of a coagulation cascade for clot formation that involves platelets and fibrin. Fibrin clots exert a hemostatic effect that not only provides a temporary extracellular matrix (ECM) for cell migration but also releases bioactive factors that recruit inflammatory cells to the injury site [2]. Neutrophils recruited to the injury site eradicate necrotic tissues and bacteria, whereas monocytes differentiate into macrophages that serve as phagocytes and promote the release of inflammatory cytokines that trigger a local immune response [3]. After the inflammatory phase, the wound healing process enters the proliferative phase that involves the restoration of the vascular network, formation of granulation tissues and epithelial regeneration. Macrophages act as important sources of growth factors and secrete vascular endothelial growth factor (VEGF) to induce vascular remodeling via endothelial cells. Fibroblasts migrate to the wound and synthesize collagen, fibronectin and other substances that promote ECM synthesis as well as form granulation tissues with new capillaries. The proliferation of keratinocytes and their migration from the wound edges to the wound center is important for the process of covering the wound (re-epithelialisation) [4]. The remodeling phase is the final stage of wound healing and is characterized by replacement of type III collagen with type I collagen in granulation tissues and fibroblasts with myofibroblasts to further seal the wound. Additionally, degradation of granulation tissues and degeneration of blood vessels render the mature wound avascular and acellular [5].

Various factors, such as hypoxia, infections, malnutrition, age, diabetes and obesity, can result in poor wound healing by affecting the multiple phases involved [6]. Poor wound healing is primarily observed in chronic refractory wounds and is characterized by the formation of hypertrophic scars that seriously affect the prognosis of wound healing, cause persistent infections and affect the appearance and normal functions of the skin, thereby imposing huge psychological and economic burdens on patients [7, 8].

Autophagy

Definition and classification of autophagy The term autophagy was coined by the Nobel Laureate, Christian de Duve (discoverer of the lysosome), in 1963 to describe the presence of single- or double-membrane intracellular vesicles that contain parts of the cytoplasm and organelles in various states of disintegration. As research to unravel autophagy has intensified over the past decades, we have a clearer understanding of the mechanism. Autophagy is a lysosome-dependent, self-renewal mechanism by which superfluous or potentially dangerous cytoplasmic cargos (e.g. damaged mitochondria, invading pathogens) are delivered to and degraded in the lysosome, with the degradation products transported back to the cytoplasm and recycled for different cellular purposes [9–11]. Autophagy is morphologically divided into three categories: macroautophagy, microautophagy and chaperone-mediated autophagy [12]. Numerous recent studies related to autophagy have primarily focused on macrophages, an area commonly referred to as macroautophagy, which is also the focus of this review. Autophagy is also categorized into non-selective and selective types. Previous studies have considered autophagy to be a non-selective biological process in cells; however, recent studies suggest that selective autophagy might be ubiquitous in cells [13]. Under normal physiological conditions, autophagy is a cellular self-defense mechanism, with a moderate level of autophagy conducive to maintaining a stable intracellular environment and coping with adverse environments. However, excessive autophagy under pathological conditions can lead to excessive degradation of cellular contents and trigger a form of cell death known as 'autophagic cell death', [14] which is considered as type II programmed cell death.

Autophagic processes and their regulation Autophagy is a dynamically balanced process referred to as autophagic flux, which includes autophagy induction, autophagosome formation, autophagolysosome formation and autophagosome degradation [14]. The processes and regulation of autophagy are shown in Figure 1. Autophagy is primarily characterized by the formation of double-membrane autophagosomes that are regulated by the products of various autophagy-related genes, among which the Unc-51-like autophagy-activating kinase 1 (ULK1) complex is the main factor that regulates autophagy initiation [15]. The phosphatidylinositol 3-kinase catalytic subunit type 3 (PI3KC3) complex, which is activated by the ULK1 complex, is another important factor involved in autophagosome formation and primarily mediates autophagosome nucleation and the recruitment of other autophagy-related proteins to autophagosome

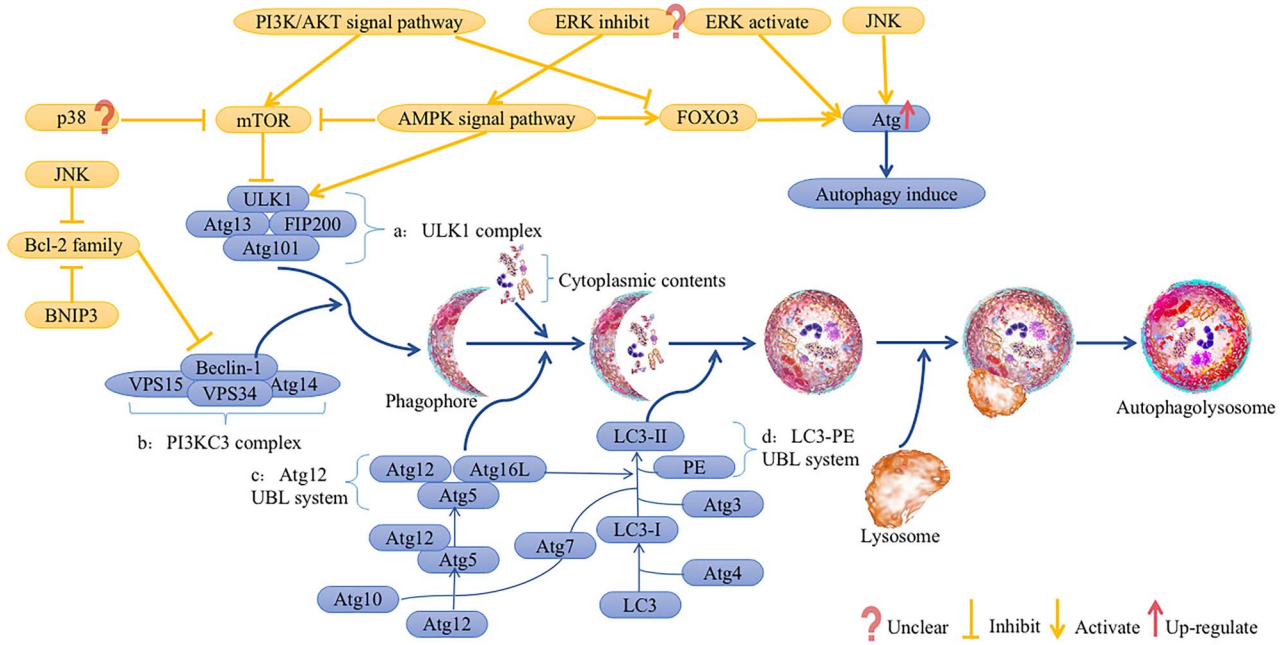

Figure 1. Autophagy processes and regulation. ULK1 complex (**a**) regulates autophagy initiation. PI3KC3 complex (**b**) primarily mediates autophagosome nucleation and the recruitment of other autophagy-related proteins to autophagosome membranes. Atg12 UBL system (**c**) and LC3-PE UBL system (**d**) are involved the elongation and maturation of autophagosome membranes. Many signaling molecules, including mTOR, BECN1, AMPK, MAPK and p38, regulate autophagy, with mTOR playing a central role in various signaling events and the PI3K/AKT/mTORC1 pathway inhibiting autophagy. Conversely, AMPK and p38 upregulate autophagy by inhibiting mTOR. The mTOR-independent pathway primarily includes the Bcl-2/BECN1 pathway, which negatively regulates autophagy; however, JNK and BNIP3 disrupt the Bcl-2–BECN1 interaction, thereby initiating autophagy. *mTOR* Mammalian target of rapamycin, *BECN1* beclin-1, *MAPK* mitogen-activated protein kinase, *PI3K* phosphatidylinositol 3-kinase, *AKT/PBK* protein kinase B, *ERK* extracellular signal-regulated kinase, *JNK* c-Jun N-terminal kinase, *mTOR* mammalian target of rapamycin, *AMPK* AMP-activated protein kinase, *FOXO3* Forkhead box O3, *Atg* autophagy-related genes, *Bcl-2* B-cell lymphoma-2, *BNIP3* BCL2/adenovirus E1B 19-kDa-interacting protein 3, *ULK1* Unc-51-like autophagy-activating kinase 1, *PI3KC3* phosphatidylinositol 3-kinase catalytic subunit type 3, *UBL* ubiquitin-like, *PE* phosphatidylethanolamine

membranes [16, 17]. Additionally, the elongation and maturation of autophagosome membranes involve two ubiquitin-like conjugation systems, Atg12–Atg5–Atg16L1 and LC3-phosphatidylethanolamine [18].

Autophagy is co-regulated by various factors and signaling pathways. In the presence of sufficient energy supply, the mammalian target of rapamycin complex 1 (mTORC1) is the major negative regulator that inhibits autophagy induction by blocking the binding between AMP-activated protein kinase (AMPK) and ULK1 via phosphorylation [19–21]. Under starvation conditions, AMPK triggers autophagy by directly activating ULK1 or inhibiting mTORC1 activity [22]. In addition to its role in the formation of the PI3KC3 complex, beclin-1 (BECN1) participates in regulating autophagy. B-cell lymphoma-2 (Bcl-2) family proteins are anti-apoptotic proteins that share the same domain as beclin-1 (the BH3 domain), through which Bcl-2 binds BECN1 and reduces its affinity to VPS34, thereby inhibiting the onset of autophagy [23]. BCL2/adenovirus E1B 19-kDa-interacting protein 3 (BNIP3) is a member of the Bcl-2 subfamily and contains only the BH3 domain, through which it induces the release of BECN1 by disrupting the Bcl-2–BECN1 interaction to initiate autophagy [24]. Forkhead box O3 (FOXO3) is another important transcription factor that participates in multiple cellular functions and is indirectly involved in autophagy by regulating the expression of autophagy-related genes via various signaling pathways [25–28]. In addition to these pathways, the mitogen-activated protein kinase (MAPK) pathway is important for autophagy regulation. MAPK signaling represents a relatively complex pathway primarily mediated by the c-Jun N-terminal kinase (JNK), p38 and extracellular signal-regulated kinase (ERK) subfamilies. Studies have shown that the JNK signaling pathway triggers autophagy in various manners [29], and the direction of p38-mediated regulation of autophagy varies across different cell types [30, 31]. Additionally, autophagy can be upregulated regardless of whether the ERK signaling pathway is activated or inhibited [32, 33]. Therefore, the mechanism by which MAPK signaling regulates autophagy requires further investigation.

Methods of assessing autophagy Identification of autophagic structures by transmission electron microscopy (TEM) is the most conventional and straightforward method. However, the labour-intensive sample-preparation procedures and challenges associated with the correct identification of autophagic structures by TEM make it a challenging procedure. LC3-II is a marker of autophagosomes, and the degradation of P62 (SQSTM1) during autophagy is considered a surrogate biomarker. Therefore, analyzing the expression of autophagy-related proteins (LC3, P62) using western blot or immunofluorescence analysis and quantifying LC3-II by flow cytometry are the commonly used methods

for evaluating autophagy. However, the amount of LC3-II or p62 at a single time-point does not reflect autophagic flux. As a result, these methods are recommended for use in combination with autophagy-related drugs to aid the detection of autophagic flux. Additionally, the development of tandem fluorescence-labeled probes, such as GFP-LC3 or mRFP/mCherry-GFP-LC3B, has been widely applied for *in vitro* studies of autophagy. However, these probes are unsuitable for the clinical analysis of autophagy in patient tissues. Therefore, there is no single method suitable for all situations, making the application of multiple assays necessary whenever possible to accurately monitor autophagy [34, 35].

Autophagy and skin wound healing

Autophagy exerts anti-inflammatory and anti-infective activities in wounds Autophagy participates in regulating wound healing at various stages (Figure 2). Neutrophils and monocytes are recruited to an injury site during the inflammatory phase after wounding, at which time neutrophils exert their antimicrobial and proinflammatory effects primarily via phagocytosis, the production of reactive oxygen species (ROS), degranulation and the release of neutrophil extracellular traps (NETs) [36]. Numerous studies have demonstrated that autophagy is closely associated with neutrophil-specific biological functions. For example, *in vitro* infection of human neutrophils with *Streptococcus pneumoniae* induces autophagy and enhances their phagocytic activity, whereas exposure to autophagy inhibitors substantially reduces the rate of phagocytosis [37]. Moreover, other studies have found that mice with defects in certain autophagy-related genes (*Atg5/7*) exhibit markedly reduced levels of degranulation and ROS in their neutrophils [38]. Additionally, autophagy induction improves the production of NETs and the survival rate of mice with sepsis [39].

Macrophages are heterogeneous cells capable of differentiation into various phenotypes in different microenvironments (M1 macrophages kill pathogens and promote the release of inflammatory factors during the early inflammatory phase, whereas M2 macrophages promote tissue repair by suppressing immune responses during the late inflammatory phase) [40]. A previous study has found that both *in vitro* and *in vivo* treatment of macrophages with the autophagy inhibitor 3-methyladenine enhances their phagocytic activity against pathogens [41]. Additionally, autophagy dysfunction in macrophages promotes their polarization towards the M1 phenotype, whereas autophagy induction promotes polarization towards the M2 phenotype to alleviate inflammatory responses and promote tissue repair [42–44]. However, the mechanism by which autophagy regulates the macrophage phenotype remains inconclusive. Therefore, further studies are required to investigate the relationship between autophagy and macrophages.

In addition to modulating innate immune cells, autophagy directly eradicates pathogens. Autophagy adaptors, such as P62 (SQSTM1), NDP52, OPTN and NBR1, recognize and recruit intracellular ubiquitinated pathogens to autophagosomes for xenophagy [45]. However, macrophages and other immune cells recognize pathogen-associated molecular patterns and damage-associated molecular patterns of pathogens via pattern-recognition receptors (PRRs) to activate innate immune responses and induce autophagy [46]. Of the two common PRRs [Toll-like receptors (TLRs) and Nod-like receptors], TLRs induce autophagy by activating the TGF-β-activated kinase (TAK1)–AMPK axis [47]. The current understanding of xenophagy is limited to autophagy induction by pathogens; therefore, the detailed degradation mechanism requires further exploration.

In summary, activation of autophagy facilitates the activation of neutrophils and enhances their immune activity. Additionally, autophagy activation contributes to the conversion of macrophages into the M2 type, which play a role in immunosuppression and promotion of tissue repair. Furthermore, autophagy plays a vital role against infections by directly or indirectly enhancing pathogen clearance.

Autophagy promotes re-epithelialisation and angiogenesis in wounds To determine the role of autophagy in keratinocyte proliferation and differentiation, Lei Qiang *et al*. [48] found that keratinocytes in the wound neoepidermis of *Atg5/7*-knockout mice showed lower proliferation and differentiation rates than those in wild-type mice. PDZ-binding kinase (PBK) promotes cell proliferation by mediating p38 activation. A previous study showed that *PBK* knockdown inhibits the proliferation of human epidermal keratinocytes, and that loss of *BECN1* downregulates the expression of *PBK*, suggesting that BECN1 affects the proliferation of human epidermal keratinocytes by regulating PBK signaling [49]. Additionally, Zhang *et al*. [50] demonstrated that a hypoxic microenvironment formed during the early stage of injuries induces the production of abundant ROS, which activates p38 and JNK–MAPK signaling and upregulates BNIP3-mediated autophagy to promote keratinocyte migration.

Autophagy induction in endothelial cells not only promotes apoptosis resistance and cell survival but also protects endothelial cells against oxidative damage [51, 52]. A previous report revealed that intermittent hypoxia induces autophagy via the AMPK–mTOR signaling pathway to reduce the apoptosis rate and improve the function of human umbilical vein endothelial cells (HUVECs) [53]. Additionally, FOXO3a improves the function of endothelial progenitor cells (EPCs) via autophagy [54]. Recent studies suggest that hypoxia-induced autophagy serves not only as a protective mechanism for endothelial cells but also as an inducer of angiogenesis [55, 56]. Treatment with autophagy inhibitors or downregulation of autophagy by silencing *Atg5* expression markedly inhibits the migratory and tube-forming activities of endothelial cells [57]. Chandel *et* al. [58] confirmed that the protein tyrosine phosphatase, which contains proline-glutamate-serine-threonine as a potential major factor, is associated with inducing autophagy and angiogenesis by regulating hypoxia-activated AMPK signaling in endothelial cells.

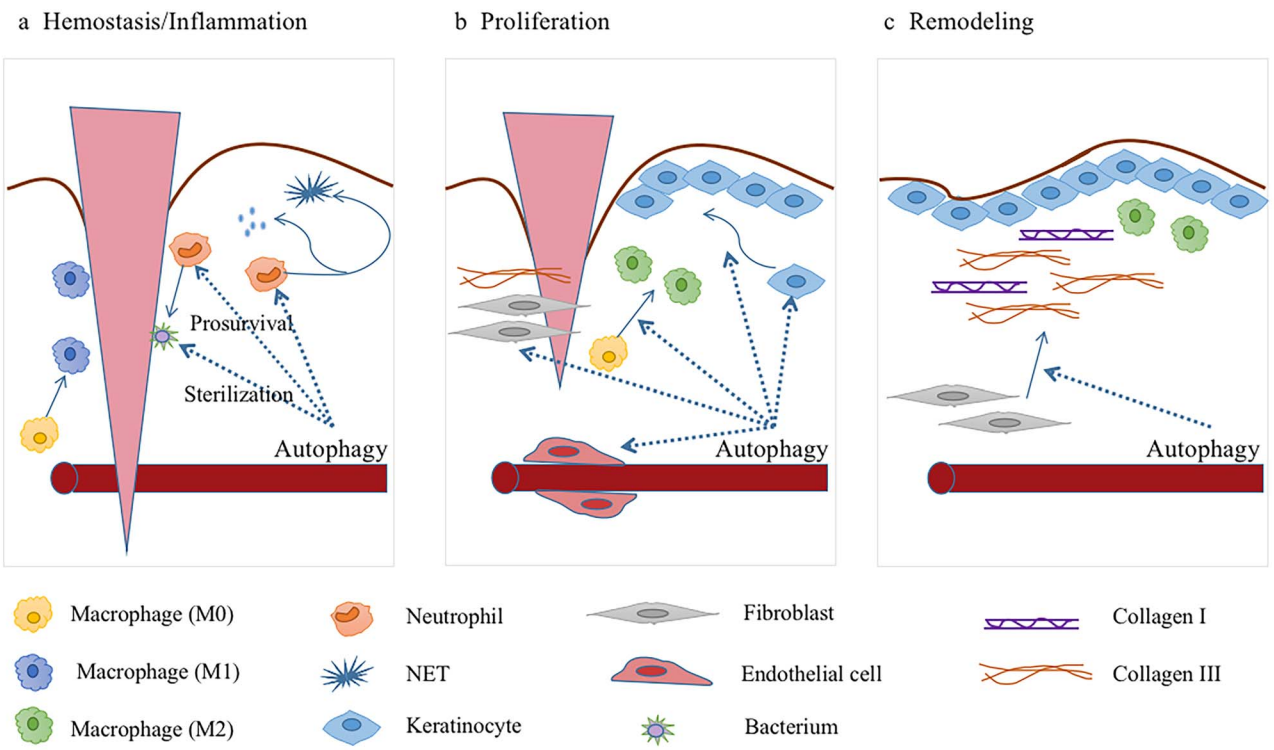

Figure 2. The role of autophagy in cutaneous wound healing. Autophagy participates in regulating wound healing at the haemostasis/inflammatory (**a**), proliferative (**b**), and remodelling (**c**) phases. Autophagy promotes the survival, proliferation and migration of neutrophils, macrophages, endothelial cells, keratinocytes and fibroblasts, which facilitates their biological functions and promotes wound healing. *NET* neutrophil extracellular trap

These findings indicate that autophagy promotes the differentiation, proliferation and migration of keratinocytes during the process of wound re-epithelisation by suppressing apoptosis and oxidative stress injury and promoting HUVEC survival and angiogenesis.

Autophagy facilitates the repair and reconstruction of damaged tissues Fibroblasts play an important role in both the proliferative and remodeling phases of wound healing. Zhou *et al*. [59] found that the transcription factor EB plays a major role in regulating autophagy, whereby it mediates autophagy to ensure fibroblast survival and maintain their functions. Additionally, remifentanil is a common, short-acting, synthetic opioid analgesic drug that inhibits oxidative stress-induced apoptosis of skin fibroblasts by activating autophagy [60]. Moreover, numerous studies have shown that autophagy activation and inhibition are closely related to fibroblast differentiation [61]. For example, observation of spatiotemporal changes in LC3-positive dots in fibroblasts and myofibroblasts in a rat model of wound healing revealed substantial increases in the number of LC3-positive dots during the late proliferative phase. Notably, their number was higher at the edge rather than the center of the wound, suggesting that the fibroblasts in the margin were in the differentiation phase [62]. Interestingly, a previous study found that gingival wound healing does not involve the autophagic process, resulting in less myofibroblast differentiation and minimal scar formation. Conversely, inflammation induces autophagy, which in turn continuously activates myofibroblasts and leads to cicatrix healing of oral mucosa [63]. Thus, autophagy exerts dual effects on the regulation of wound healing and can determine different clinical outcomes depending on the tissue or cell in which it occurs.

In addition to the wound healing response, tissue dysfunction after injury is readily evident in skin but also occurs internally across organ systems in the form of fibrosis. Skin scarring is the most common chronic fibrotic disease. A previous study reported that hypertrophic scar tissues and fibroblasts show higher levels of autophagy than normal skin [64]. Autophagy downregulation in fibroblasts induces apoptosis, thereby degrading excessive intracellular fibrin, improving excessive deposition of ECM and inhibiting fibrosis progression [65]. However, some studies have also shown that excessive autophagy stimulates fibroblast apoptosis and autophagic death, inhibits their proliferation and improves the formation of hypertrophic scars [66, 67]. These findings suggest that autophagy in fibroblasts accelerates wound healing and facilitates scar formation. Similar processes are also observed in other fibrotic diseases. For example, upregulation of autophagy aggravates liver fibrosis by promoting hepatic stellate cell activation or inhibits liver fibrosis by protecting damaged hepatocytes [68, 69]. Additionally, defective autophagy results in the deposition of ECM to promote the development of pulmonary fibrosis [70]. Therefore, autophagy is expected to become a new target for the treatment of skin scars and skin fibrosis.

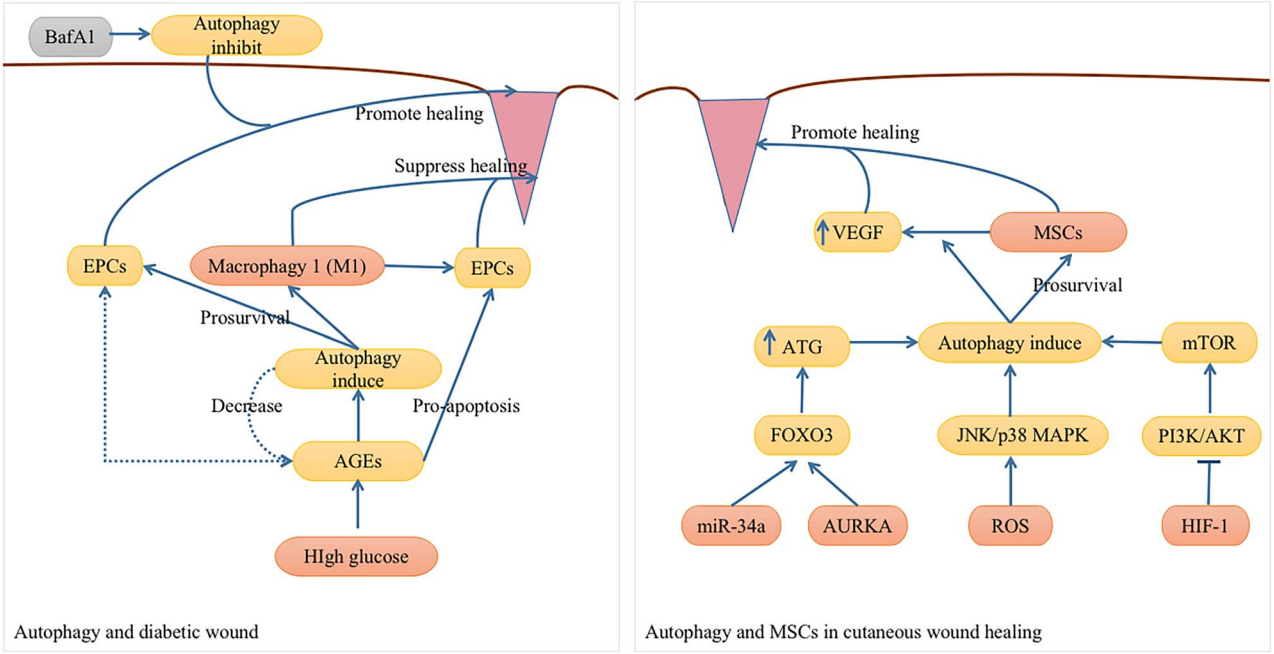

Figure 3. Autophagy, mesenchymal stem cells and diabetic wounds. In diabetic wounds, autophagy protects endothelial cells against damage caused by AGEs. However, it can also delay healing by affecting macrophage polarization (left). Moderately regulated autophagy improves the survival rate of MSCs after transplantation (right). *BafA1* bafilomycin A1, *EPC* endothelial progenitor cells, *AGEs* advanced glycation end-products, *VEGF* vascular endothelial growth factor, *MSCs* mesenchymal stem cells, *Atg* autophagy-related genes, *mTOR* mammalian target of rapamycin, *FOXO3* Forkhead box O3, *JNK* c-Jun N-terminal kinase, *MAPK* mitogen-activated protein kinase, *PI3K* phosphatidylinositol 3-kinase, *AKT/PBK* protein kinase B, *AURKA* aurora kinase A, *ROS* reactive oxygen species, *HIF-1* hypoxia-inducible factor 1

Autophagy and chronic wounds associated with diabetes

Research progress on the relationship between autophagy and diabetic wound healing is shown in Figure 3. Numerous studies suggest that dysregulation of autophagy is a factor in diabetic refractory wounds. Additionally, a hyperglycemic environment markedly impairs the migratory capacity of keratinocytes, likely by inhibiting the activation of p38 MAPK signaling and downregulating the autophagy-related proteins Atg5 and LC3-II, thereby inhibiting autophagy and decelerating cell migration [71]. Advanced glycation end-products (AGEs) are the final products produced by non-enzymatic reactions between the amino groups of proteins, nucleotides and nucleic acids and the aldehyde groups of sugars under persistent hyperglycemic conditions [72]. Numerous studies have confirmed that AGEs cause refractory wounds by affecting the functions of multiple cell types, and that autophagy plays an important role in AGE-induced refractory wounds. A previous study reported that AGEs cause refractory wounds by promoting macrophage polarization towards the M1 phenotype via autophagy activation [73]. Another report indicated that melatonin therapy not only reduces AGE-induced apoptosis of EPCs but also enhances autophagic flux in these cells, thereby promoting their migration and adhesion [74]. Additionally, excessively accumulated AGEs can be eliminated via p62 (an autophagy receptor)-dependent autophagy to reduce their cytotoxicity [75, 76].

Many mechanisms of autophagy in diabetic wound healing have been revealed; however, the interaction of multiple mechanisms and the specific mechanism associated with how cells perform their biological functions by constantly adjusting their autophagic activity in diabetic wounds remain unclear. Nevertheless, recent studies have shown that refractory diabetic wounds may be related to an increase in autophagy. Therefore, downregulating autophagy levels in diabetic wounds may be a promising therapeutic strategy.

Autophagy regulates the survival and secretory functions of mesenchymal stem cells

In recent years, studies have shown that mesenchymal stem cells (MSCs) play essential roles in tissue regeneration and wound repair by promoting angiogenesis [77], regulating inflammatory responses [78] and enhancing re-epithelialisation [79]. However, wound microenvironments can affect MSC functions and increase their apoptotic rate, which results in low survival rates of transplanted MSCs [80].

Research progress on the regulatory role of autophagy in MSCs is shown in Figure 3. Previous studies have demonstrated that regulating autophagy might be an effective strategy to promote MSC survival and improve wound healing outcomes [81–83]. A previous study found that palmitate promotes MSC apoptosis by inducing intracellular accumulation of ROS, whereas autophagy induction via the ROS–JNK–p38 MAPK signaling pathway protects MSCs against apoptosis [84]. Hypoxia-inducible factor-1α overexpression promotes MSC survival under hypoxic conditions by inducing

autophagy via inhibition of PI3K/AKT/mTOR signaling [85]. Additionally, the serine/threonine kinase aurora kinase A induces autophagy by targeting FOXO3a to protect adipose-derived stem cells against hyperglycemia-induced apoptosis [86]. Similar studies found that inhibition of microRNA (miR)-34a improves the therapeutic application of MSCs against diabetic wounds by activating sirtuin-1/FOXO3a pathway-mediated autophagy [87]. Additionally, An *et al*. [77] showed that subcutaneous injection of MSCs pretreated with autophagy inducers promotes the secretion of VEGF by activating MSC-specific paracrine signaling through the ERK1/2 pathway and subsequently enhances wound healing.

The regulation of autophagy plays a significant role in MSC survival under high-stress conditions following transplantation. Therefore, fine-tuned regulation of autophagy in MSCs may be important for their clinical application in the treatment of wound healing.

miRNAs participate in autophagy regulation during wound healing

Numerous recent studies have confirmed the involvement of miRNAs in regulating multiple processes in wound healing [88, 89], as well as playing a pivotal regulatory role in autophagy [90]. Therefore, studies on miR-mediated regulation of autophagy to affect wound healing have received increasing attention. A previous study has shown that *BNIP3* overexpression promotes the proliferation and migration of human keratinocytes, with both bioinformatics analysis and experimental results subsequently confirming a negative correlation between miR-96-5p expression and the levels of its direct downstream target (*BNIP3*), as well as the proliferation and migration of human keratinocytes [91]. Additionally, Zeng *et* al. [92] found that miR-106b-5p was substantially upregulated in exosomes of AGE-pretreated HUVECs, and that miR-106b-5p induced autophagy in fibroblasts by inhibiting ERK1/2 expression, thereby reducing collagen synthesis and delaying the wound healing process. Moreover, Wang *et al*. [93] demonstrated that miR-103/107 positively regulates the late stage of autophagy in human epidermal keratinocytes. Furthermore, eradication of pathogenic bacteria via autophagy during infections and inflammation is modulated by regulating the expression levels of various miRNAs [94]. Currently, studies on miR-mediated regulation of autophagy to improve wound healing remain relatively limited; therefore, further studies are warranted to clarify the mechanism of action that underlies the relationships between miRNAs, autophagy and wound healing in order to identify new therapeutic strategies that promote wound healing.

Therapeutic potential of targeting autophagy in wound healing

Autophagy is an evolutionarily conserved process by which long-lived proteins and organelles are sequestered by autophagosomes and subsequently degraded by lysosomes for recycling. In recent years, increasing numbers of researchers have found that autophagy also plays a crucial role in wound healing. Thus, modulating autophagy represents an attractive future therapeutic target for treating wounds. There are several autophagy inhibitors and activators in development or clinical trials already that may be useful to promote skin wound healing. For example, a previous report revealed that treatment of wounds with the autophagy inhibitor bafilomycin A1 in diabetic mice substantially accelerated wound healing with an excellent healing outcome [95]. Rapamycin activates autophagy by targeting mTORC1, thereby regulating the proliferation and migration of fibroblasts from injured wounds [96]. However, bafilomycin A1, a potent and specific inhibitor of Vacuolar H(+)-ATPase (V-ATPase), exerts severe and acute toxic reactions when administered to animals, and rapamycin could also influence many other metabolic pathways. Therefore, the application of these autophagy regulators is still limited in the clinical setting. Additionally, monotropein (a bioactive constitutional used in traditional Chinese medicine) and noncoding RNA have also been confirmed to promote wound healing by regulating autophagy [97, 98]. Moreover, MSCs have shown promising results for repairing damaged tissues both in animal models and in human clinical trials and may become an ideal autophagy inducer in the promotion of wound healing [83]. It has been reported that MSCs enhanced autophagy and thereby protected cells against chronic high glucose-induced injury *in vitro* [99]. Exosomes derived from mmu_circ_0000250-modified adipose-derived stem cells (ADSCs) accelerate wound healing in diabetic mice by promoting autophagy activation [100]. Furthermore, since protein kinases are integral to the autophagy process, it is critically important to understand the role of kinases in autophagic regulation. At present, intervention in autophagic processes with small-molecule modulators targeting specific kinases has becoming a reasonable and prevalent strategy for treating several varieties of human diseases, including wounds. Although targeting autophagy in the treatment of skin wounds has achieved limited results, the dual effects of autophagy require further investigation to confirm its potential clinical efficacy.

Conclusions

Autophagy plays a complex role in wound healing by facilitating the activation of inflammatory cells and enhancing their anti-inflammatory and anti-infective activities. Additionally, autophagy is conducive to the survival, migration and proliferation of cells associated with wound healing, although excessive autophagy can promote the formation of hypertrophic scars. Furthermore, enhanced autophagy impairs the healing process of diabetic wounds while also protecting cells against damage caused by AGEs and enhancing the wound repair ability of stem cells. Therefore, it is necessary to clarify the specific role of autophagy in each stage of wound healing and develop autophagy drugs to promote wound healing by targeting specific cells. Regulation

of autophagy has achieved promising results in animal wound healing, including diabetic skin ulcers. Therefore, it is believed that an in-depth understanding of the autophagy mechanism will promote fine-tuned regulation of autophagy in cells and tissues and potentially provide a new therapeutic strategy for refractory wounds.

Abbreviations

AGEs: Advanced glycation end-products; AMPK: AMP-activated protein kinase; Atg: Autophagy-related genes; Bcl-2: B-cell lymphoma-2; BECN1: Beclin1; BNIP3: BCL2/adenovirus E1B 19-kDa-interacting protein 3; ECM: Extracellular matrix; EPC: Endothelial progenitor cells; ERK: Extracellular signal-regulated kinase; FOXO3: Forkhead box O3; HUVEC: Human umbilical vein endothelial cells; JNK: c-Jun N-terminal kinase; MAPK: Mitogen-activated protein kinase; miR: microRNA; MSCs: Mesenchymal stem cells; mTOR: Mammalian target of rapamycin; NET: Neutrophil extracellular trap; PBK: PDZ-binding kinase; PI3KC3: Phosphatidylinositol 3-kinase catalytic subunit type 3; PRR: Pattern-recognition receptors; ROS: Reactive oxygen species; TEM: transmission electron microscopy; TLR: Toll-like receptor; ULK1: Unc-51-like autophagy-activating kinase 1; VEGF: vascular endothelial growth factor.

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Authors' contributions

HR is the main writer of the review, completing the collection and analysis of relevant literature and the writing of the first draft of the paper. QZ and XH participated in the analysis and collation of literature. FZ and ZW are the project's framers and principals, guiding the writing of the thesis. All authors read and approved the final manuscript.

Conflicts of interests

None declared.

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