Extracellular Vesicles in Idiopathic Pulmonary Fibrosis Progression

Definition

Idiopathic pulmonary fibrosis (IPF) is a fibrosing interstitial lung disease of unknown etiology. Different types of cells are involved in fibrogenesis, which is persistently physical and molecular stimulation, either directly or by interacting with bioactive molecules and extracellular vesicles (EVs). Current evidence suggests that EVs play an essential role in IPF development. EVs are released by a variety of cells, including fibroblasts, epithelial cells, and alveolar macrophages. In addition, EVs can transport bioactive molecules, such as lipids, proteins, and nucleic acids, which play a pivotal role in cellular communication. Several proposed mechanisms show that an acceptor cell can capture, absorb, or interact with EVs through direct fusion with the plasma membrane, ligand-receptor interaction, and endocytotic process, modifying the target cell. During fibrogenesis, the release of EVs is deregulated, increases the EVs amount, and the cargo content is modified. This alteration is closely associated with the maintenance of the fibrotic microenvironment.

1. Idiopathic Pulmonary Fibrosis

Idiopathic pulmonary fibrosis (IPF) is fibrosing interstitial pneumonia of unknown etiology. It is a chronic and progressive disease characterized by aberrant deposition of extracellular matrix (ECM) in the lung parenchyma that deforms the alveolar architecture, causing respiratory failure as it decreases lung function and gas exchange, which can eventually cause death [1]. The synergistic effect of both internal and external factors are required for the initiation and progression of IPF mainly, in subjects with a genetic predisposition. Tissue alterations are established through recurrent lesions in the pulmonary epithelium superimposed on the accelerated aging of the epithelium itself, leading to an aberrant repair of lung parenchyma and causing excessive accumulation of ECM and all clinical features of the disease [1][2].

2. EVs and Their Involvement in Cellular and Molecular Mechanisms Associated with IPF

Several reports have proposed that EVs function as messengers and critical players in the pathogenesis of IPF [3]. However, the mechanism promoting lung fibrogenesis remains to be fully described. Therefore, further researches are still needed to elucidate both cellular and molecular mechanisms involved in this process. Below is a brief summarization of the current evidence on the involvement of EVs secreted by cells participating in the development of IPF (Figure 1).
Figure 1. Schematic representation of EVs released by different lung cells involved in Idiopathic pulmonary fibrosis (IPF) pathogenesis. EVs and their cargo molecules regulate the main signaling pathways associated with profibrotic processes facilitating the IPF progression.

2.1. Cells Involved in IPF Development

The involvement of cells in the fibrogenesis of IPF has been extensively studied; nevertheless, the exact mechanism of EVs in promoting cellular communication leading to IPF development and evolution remains unclear. Current knowledge on the pathogenesis of IPF has demonstrated that during the early stage of the disease, chronic tissue injury promote apoptosis of type I alveolar epithelial cell (AEC-I), recruitment of inflammatory cells, mainly macrophages and neutrophils, activation of type II alveolar epithelial cell (AEC-II) that regenerate damaged cells, and the release of proinflammatory cytokines that promote the recruitment, activation, and proliferation of fibroblasts. Thus, fibroblasts are the most important cells in IPF development since they undergo differentiation into myofibroblasts facilitating their capability to produce and secrete ECM components.

2.1.1. EVs Released by Alveolar Epithelial Cells

A critical step during IPF development is the persistent injury of AEC-I and II by environmental factors. During this process, AECs may undergo apoptosis, release inflammatory mediators. EVs that strongly contribute to IPF development. Recently, it has been reported that syndecan-1 protein is increased in both lung epithelium of IPF patients and murine bleomycin-induced IPF models. Likewise, authors also demonstrated that syndecan-1 plays an essential role in the packaging of antifibrotic miRNAs in EVs, including miR-144-3p, miR-142a-3p, miR-142b, miR-503-3p, and miR-34b-5p, which have shown diverse effects on signaling pathways associated with fibroproliferation and fibrogenesis, suggesting that those miRNAs play a pivotal role in the regulation of IPF progress. Moreover, they observed that by incubating AEC-II with EVs isolated from wild-type and Sdc1−/− mice models of IPF induced with bleomycin, EVs from wild-type mice act as a profibrotic signal promoting the activation of different signaling pathways involved in fibrotic processes, such as TGF-β and Wnt as compared with the effect of EVs isolated from Sdc1−/− mice.

2.1.2. EVs Secreted by Macrophages
Alveolar macrophages secrete EVs to transport the suppressor of cytokine signaling 1 and 3 (SOCS1 and SOCS3, respectively). Consequently, they are primarily taken up by AECs, resulting in the inhibition of signal transducer and activator of transcription (STAT), which regulates the inflammatory response, indicating that EVs derived from macrophages are involved in the pulmonary homeostasis.

On the other hand, Yao et al. have demonstrated that macrophage-derived EVs bearing an M2 phenotype transport miR-328 and that this miRNA charge participates in promoting IPF progression by negatively regulating family with sequence similarity 13, member A (FAM13A) and as a result, inducing lung fibroblasts proliferation and overexpression of collagens 1A, 3A, and alpha-smooth muscle actin (α-SMA). Recently, it has been demonstrated that EVs secretion increases in the supernatant of silica (SiO2)-stimulated macrophage. Moreover, lung fibroblasts incubated with these EVs are differentiated into myofibroblasts by inducing endoplasmic reticulum stress, which results in a significant increase in proliferation, migration, and expression of fibrotic proteins, such as collagen I and α-SMA. Besides, it was reported that miR-125a-5p is upregulated in EVs of silica (SiO2)-stimulated macrophage, and when lung fibroblasts are incubated with these EVs, α-SMA is overexpressed, and TGF-β is activated to mediate their differentiation into myofibroblasts through downregulation of Smurf1.

Contrarily, it has been described that miR-142-3p contained in EVs derived from macrophages are responsible for suppressing the profibrotic activation of both AECs and lung fibroblasts mediated by TGF-β as well as of repressing the expression of profibrotic genes, such as COL1A1, COL1A2, and TGF-β.

2.1.3. EVs Released by Fibroblasts

Fibroblasts are in charge of restoring the ECM components after injury; nonetheless, in the pathogenesis of IPF, this capability is altered by the persistent exposition to profibrotic factors, which leads to increased production of ECM components. Circulating proteins, such as TGF-β and PDGF, have been described as the main profibrotic factors in the fibroblasts activation process, and the role of EVs as carriers of signals promoting fibroblasts activation has also been studied. Martin-Medina et al. demonstrated that Wnt family member 5A (WNT5A) protein is overexpressed in EVs isolated from both BALF of IPF patients and an IPF bleomycin-induced murine model. Furthermore, they reported that TGF-β-mediated activation of primary human pulmonary lung fibroblasts (PHLF) is closely associated with an increased level of WNT5A and its release within EVs. They also demonstrated that EVs isolated from BALF of IPF patients and cell culture supernatant of PHLF activated with TGF-β increased lung fibroblasts proliferation in a WNT5A-dependent manner.

Alternatively, fibroblasts from IPF patients have been reported to secrete EVs fibronectin-enriched, which promote an invasive phenotype in recipient fibroblasts through the interaction of fibronectin with α5β1 integrin, triggering the activation of signaling pathways associated with cell invasion processes, such as Src family kinases and focal adhesion kinase.

A recent study has demonstrated that after stimulation of human and murine lung fibroblasts with TGF-β, programmed death-ligand 1 (PD-L1) is overexpressed in EVs derived from fibroblasts; PD-L1 acts as a paracrine mediator of the immunosuppression by reducing T-cell proliferation while promoting fibroblasts migration. Another investigation group has demonstrated that EVs secreted by lung fibroblasts isolated from IPF patients promote mitochondrial damage and senescence of human bronchial epithelial cells (HBECs). In addition, they evidenced that these EVs contain a high amount of miRNAs, including miR-23b-3p and miR-494-3p, that suppress sirtuin 3 (SIRT3) expression, thus favoring epithelial phenotypic changes.

3. Involvement of MSCs in Lung Repair

Previous studies have proposed that mesenchymal stem cells (MSCs) are responsible for maintaining vascular homeostasis and facilitating repair; contrarily, studies performed in lung tissue have reported that MSCs participates in the restoration of injured endothelium through the release of paracrine...
mediators, such as EVs and activation of different signaling pathways, including (IL-6), interleukin 6 receptor subunit alpha (IL-6RA), Janus kinase (JAK), signal transducer and activator of transcription 3 (STAT3) [17][18]. In vitro studies demonstrated that human lung resident MSCs (Lr-MSCs) can decrease fibroblasts proliferation and increase the ability to induce wound closure in alveolar epithelial cells A549 [19], and decrease collagen deposition, T-cell, granulocyte and B-cell infiltration into the alveolar space, and limit T-cell proliferation in a bleomycin-induced model of pulmonary fibrosis [20]. Another study showed that menstrual blood-derived stem cells (MenSCs) inhibit the inflammatory response after induction of lung damage with lipopolysaccharide (LPS) [21]. In this respect, recent studies have been focused on evaluating the therapeutic potential of MSCs, as well as EVs and molecules secreted by these cells during IPF [22][23].

3.1. EVs Released by MSCs as Therapeutic Mediators

Since stem cells protect the lung from damage, several types of research have been focused on studying the role therapeutic of the mesenchymal stem cell-derived EVs (MSC-EVs) of various tissues, including the umbilical cord, menstrual blood, bone marrow, placenta, or adult organs, such as the lung (Figure 2). This is because MSC-EVs have been reported to mimic the therapeutic capabilities of MSCs and maintain a reduced immunogenic property [24][25]. In this respect, it has been described that MSC-EVs might regulate proliferation, maturation, polarization, and cell migration, as well as modulate inflammatory cytokines, transcription factors, and miRNAs function in the cell microenvironment. Although the repairing mechanism has not been fully elucidated, evidence indicates that the reprogramming mechanism of lung cells induced by damage might be associated with miRNA-delivery, such as miR-186, which is contained in human bone marrow mesenchymal stem cell-derived EVs (BMSC-EV) and might reduce fibroblasts activation by downregulating SRY-box transcription factor 4 (SOX4) and Dickkopf 1 (DKK1), an inhibitor of the WNT signaling pathway, resulting in collagen reduction [24][26][27]. However, the studies and results obtained on the therapeutic potential of MSC-EVs in IPF have been studied mainly in murine and in vitro models (Table 1 and Table 2). Therefore, there are still many limitations to the possible therapeutic application of MSC-EVs in human IPF.

Figure 2. Function of stem cell-derived EVs. MSCs from either menstrual blood, bone marrow, placenta, or adult organs, such as the lung, exert a therapeutic effect through their secretome, which contains EVs that transport miRNAs that regulate several processes, including inflammation, by decreasing neutrophil recruitment, reducing cell proliferation, decreasing the apoptosis of injured epithelium and proinflammatory cytokines secretion, and reducing fibroblasts activation by transferring miRNAs.

Table 1. Summary of studies in animal models of IPF with therapeutic MSC-EVs.

<table>
<thead>
<tr>
<th>Experimental Model</th>
<th>EVs Source</th>
<th>Cargo</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radiation-induced lung</td>
<td>hP-MSCs</td>
<td>miR-214-</td>
<td>Attenuates pulmonary vascular damage, inflammation, and fibrosis.</td>
<td>[24]</td>
</tr>
<tr>
<td>injury</td>
<td></td>
<td>3p</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental Model</td>
<td>EVs Source</td>
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<td>Effects</td>
<td>Reference</td>
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<td>-----------</td>
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<tr>
<td>Bleomycin-induced IPF</td>
<td>BMSCs</td>
<td>miR-186</td>
<td>Relieves IPF by blocking fibroblasts activation by suppressing SOX4 and DKK1 expression.</td>
<td>[28]</td>
</tr>
<tr>
<td>E. coli endotoxin induced ALI</td>
<td>BMSCs</td>
<td>KGF mRNA</td>
<td>Restores lung protein permeability and reduces inflammation.</td>
<td>[27]</td>
</tr>
<tr>
<td>IRI-induced ALI</td>
<td>BMSCs</td>
<td>miR-21-5p</td>
<td>Decreases edema, pulmonary dysfunction, M1 polarization of alveolar macrophages, and secretion of the cytokines IL-8, IL-1β, IL-6, IL-17, and TNF-α.</td>
<td>[29]</td>
</tr>
<tr>
<td>Hypoxia-induced PH</td>
<td>BMSCs</td>
<td>unknown</td>
<td>Prevents activation of hypoxic signaling, lung inflammation, and PH development through inhibition of hypoxic STAT3 signaling.</td>
<td>[30]</td>
</tr>
<tr>
<td>Bleomycin-induced IPF</td>
<td>BMSCs</td>
<td>miR-29b-3p</td>
<td>Attenuates IPF progression by suppressing fibroblasts proliferation, migration, and differentiation through suppression of FZD6 expression.</td>
<td>[31]</td>
</tr>
<tr>
<td>LPS-induced ALI</td>
<td>BMSCs</td>
<td>miR-23a-3p y miR-182-5p</td>
<td>Attenuate lung injury, EMT, and fibrosis by inhibiting NF-κB and hedgehog pathways.</td>
<td>[32]</td>
</tr>
<tr>
<td>LPS-induced ALI</td>
<td>AD-MSCs</td>
<td>unknown</td>
<td>They reduce inflammation, alveolar septal thickening, alter macrophage phenotypes, reduce levels of the proinflammatory cytokine IL-1β, and increase anti-inflammatory IL-10.</td>
<td>[33]</td>
</tr>
<tr>
<td>LPS-induced ALI</td>
<td>AD-MSCs</td>
<td>miR-27a-3p</td>
<td>Alleviates lung injury, inhibits NF-κB activation and promotes M2 polarization of alveolar macrophages.</td>
<td>[34]</td>
</tr>
<tr>
<td>Bleomycin-induced IPF</td>
<td>hAECs</td>
<td>unknown</td>
<td>Attenuates inflammation and pulmonary fibrosis.</td>
<td>[35]</td>
</tr>
<tr>
<td>Bleomycin-induced IPF</td>
<td>MenSCs</td>
<td>miR-Let-7</td>
<td>Attenuates lung inflammation and fibrosis by regulating ROS, mtDNA damage, and NLRP3 inflammasome activation.</td>
<td>[36]</td>
</tr>
</tbody>
</table>

hP-MSCs = Placenta-derived mesenchymal stem cell; BMSCs = Bone marrow mesenchymal stem cell; AD-MSCs = adipose-derived mesenchymal stem cells; hAECs = Human amnion epithelial cells; MenSCs = Menstrual blood-derived stem cells; IPF = Idiopathic pulmonary fibrosis; ALI = Acute lung injury; LPS = Lipopolysaccharide; IRI = Ischemia/reperfusion injury; PH = Pulmonary hypertension; EMT = Epithelial-mesenchymal transition; ROS = Reactive oxygen species; DKK1 = Dickkopf-1; IL-8 = Interleukin-8; IL-1β = Interleukin-1β; IL-6 = Interleukin-6; IL-17 = Interleukin-17; TNF-α = Tumor necrosis factor alpha; IL-10 = Interleukin-10; STAT3 = signal transducer and activator of transcription 3; FZD6 = Frizzled-6; NF-κB = Nuclear factor kappa B; mtDNA= mitochondrial DNA; NLRP3= NLR family pyrin domain containing 3; SOX4= SRY-box transcription factor 4.

**Table 2.** Summary of studies in vitro models of IPF and other lung diseases with therapeutic MSC-EVs.

<table>
<thead>
<tr>
<th>EVs Source</th>
<th>Cargo</th>
<th>Target</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>hAECs</td>
<td>unknown</td>
<td>HLF activated with TGF-β</td>
<td>Inhibit fibroblasts activation</td>
<td>[35]</td>
</tr>
<tr>
<td>BMSCs</td>
<td>miR-186</td>
<td>Fibroblasts LL29</td>
<td>Inhibit fibroblasts activation by suppression of SOX4 and DKK1</td>
<td>[27]</td>
</tr>
<tr>
<td>BMSCs</td>
<td>interaction of Thy-1 with beta integrins</td>
<td>CCL-210 (HLF)</td>
<td>Blocks myofibroblastic differentiation</td>
<td>[27]</td>
</tr>
</tbody>
</table>
BMSCs = Bone marrow mesenchymal stem cell; AD-MSCs = adipose-derived mesenchymal stem cells; hAECs = Human amnion epithelial cells; MenSCs = Menstrual blood-derived stem cells; LPS = Lipopolysaccharide; HLF = Human Lung Fibroblasts; EMT = Epithelial–mesenchymal transition; ROS = Reactive oxygen species; DKK1 = Dickkopf-1; IL-1β = Interleukin-1β; IL-6 = Interleukin-6; IL-12 = Interleukin-12; TNF-α = Tumor necrosis factor alpha; FZD6 = Frizzled-6; NF-κB = Nuclear factor kappa B; SOX4 = SRY-box transcription factor 4; MLE-12 = murine lung epithelial cell; mtDNA = mitochondrial DNA; NLRP3 = NLR family pyrin domain containing 3.

### 3.1.1. hUMSC-EVs

MSC-EVs can reduce the recruitment of inflammatory cells, cytokine levels, and the protein concentration in alveoli, which introduce pulmonary edema and decrease intrinsic and extrinsic apoptosis pathways in the injured epithelium [27]. In addition, studies have suggested that MSC-EVs obtained from different tissues facilitate the resolution of inflammatory response and apoptosis in the lung; some models have been used to demonstrate the ability of MSC-EVs: the chronic asthma model where hUCMSC-EVs obtained under hypoxic conditions, they observed that had a greater capacity to suppress airway inflammation in asthmatic mice, as well as the decrease of profibrogenic markers such as α-SMA, collagen-1, and TGF-β-p-smad2/3 signaling pathway; in the chronic obstructive pulmonary disease model, they also found that hUMSC-EVs decreased inflammation, reduced damage to the alveolar septum, and reduced the expression levels of the proinflammatory transcription factor nuclear factor kappa B (NF-κB) [29][40][41].

### 3.1.2. hAEC-EVs

EVs derived from human amnion epithelial cells (hAECs) modulate the antifibrotic, immunomodulatory, and regenerative properties implicated in IPF initial stage; thus, regulating the response of immune cells by inducing apoptosis of neutrophils and by inhibiting the proliferation of T cells through a mechanism dependent of the transfer of anti-fibrotic proteins and miRNAs contained in EVs, despite the effect of these EVs, the molecular mechanisms remain poorly elucidated, so further studies are needed to understand which signaling pathway hAEC-EVs induce α-SMA reduction, increase the percentage of AEC-II and inhibit myofibroblasts differentiation and their immunomodulatory activity [35].
3.1.3. AD-MSCs-EVs

Various MSC-EV therapies have been investigated as potential therapeutic strategies in experimental models of lung diseases; among them, the therapeutic potential of adipose-derived mesenchymal stem cell-derived EVs (AD-MSC-EVs) has been evaluated. Recent studies demonstrated that these AD-MSC-EVs positively attenuate acute lung injury by transferring miR-27a-3p into alveolar macrophages and promoting macrophage polarization towards an M2 phenotype [38]. Moreover, MSC-EVs function might depend on cell age, although recent studies have shown that either young or aged cells have similar physical and phenotypic properties. Nevertheless, it has been demonstrated that in LPS-induced lung damage, the EVs secreted by young cells alleviate tissue injury and have greater capability to suppress the activation of proinflammatory genes, such as IL-6, IL-1β, and tumor necrosis factor alpha (TNF-α), while EVs of aged cells do not promote a protective response and do not alter macrophage phenotypes [33].

3.1.4. BMSC-EVs

Bleomycin-induced pulmonary fibrosis treated with intravenous BMSC-EVs showed a reversal of bleomycin-induced damage by decreasing collagen deposition [42]. The mechanisms remain to be elucidated; however, other studies have shown that intravenous administration of BMSC-EVs inhibits vascular remodeling and pulmonary hypertension by suppressing hypoxic STAT3 signaling [30].

Wan X et al. have reported that miR-29b-3p is overexpressed in BMSC-EVs and inhibits proliferation, migration, differentiation, and invasion of the LL29 cell line (lung fibroblasts bearing IPF cell line) by suppressing frizzled-6 (FZD6) expression and generating antifibrotic effects in vitro and in vivo [31]. Furthermore, BMSCs-EVs suppress TGF-β-induced normal and IPF fibroblasts differentiation into myofibroblasts by decreasing α-SMA, fibronectin, and collagen III expressions. Moreover, the BMSC-EVs fibroblasts uptake is due to the presence of Thy-1, a cell surface-expressed in MSC-EVs; Thy-1 interacts with the integrins β1, β3, and β5, promoting the EVs capture [37].

Some research has shown that hBMSC-EVs modulate the activity of cells involved in immune response, such as dendritic cells. The treatment of these cells with hBMSC-EVs has shown an increased production of anti-inflammatory cytokines, mainly TGF-β, and reduced synthesis of proinflammatory cytokines, such as IL-6 and IL-12, after LPS stimulation [38]. MSC-EVs inactivate NF-κB and hedgehog pathways by transferring specific miRNAs, such as miR-182-5p and miR-23a-3p, and targeting inhibitor of nuclear factor kappa B kinase subunit Beta (Ikbkb) and ubiquitin carboxyl-terminal hydrolase 5 (Usp5), which reverts EMT in LPS-treated MLE-12 cells (murine lung epithelial cell line); [32]. Zulueta et al. have demonstrated that the effects of MSC-EVs on IB3-1 cells (Adeno-associated virus-transformed human bronchial cell line) could involve peroxisome proliferator-activated receptor gamma (PPAR-γ) activation. This transcription factor interacts with NF-κB and heme oxygenase 1 (HO-1), regulating the inflammatory cascade through the nuclear translocation of NF-κB [39].

3.1.5. MenSC-EVs

Due to the effect that MenSCs have shown as therapeutic mediators in several diseases, Sun and coworkers evaluated the potential of EVs derived from menstrual blood stem cells (MenSC-EVs), and have shown a protective effect in a bleomycin-induced IPF model by reducing collagen deposition and regulating ROS activity on alveolar epithelial cells through the transport of miR-Let-7; this is possibly through inhibiting ROS/miDNA and fibrosis signaling cascades [36].

4. Conclusions and Perspectives

In conclusion, IPF is a chronic progressive pathology of unknown etiology, poor prognosis, and high mortality rate. This disease involves a heterogeneous group of cells such as AEC-I, AEC-II, macrophages, and fibroblasts that orchestrate a pathological state mediated by a microenvironment enriched with soluble factors associated with fibrosis development, such as cytokines, chemokines, and EVs production.
In recent years, it has been shown that EVs participate in the initiation, and progression of the pathophysiological mechanisms associated with IPF; for example, immunomodulation, fibroproliferation, differentiation, and mesenchymal–epithelial transition through the transfer of bioactive cargo molecules, such as proteins (PD-L1, WNT5A and Fibronectin) and miRNAs (miR-328, miR-125a-5p, miR-142-3p, miR-23b-3p and miR-494-3p). Thus, EVs can modulate the functioning of the host cells, accelerating lung damage and leading to decreased gas exchange by excessive ECM deposition. Therefore, these crucial features strongly suggest that EVs play an essential role in the pathogenesis of IPF and may be useful for achieving a better understanding of the disease and identifying candidates for biomarkers for the diagnosis, prognosis of IPF. However, there are still many challenges ahead; despite the number of available studies describing how EVs and their cargo molecules play an essential role in IPF progression, the mechanism by which these EVs regulate cellular processes involved in IPF development remains to be fully elucidated. Therefore, further exploration and new studies are needed to expand the understanding of EVs and their cargo molecules in the pathogenesis of IPF.

References

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