An Overview about Mesenchymal stem cells and Liver Fibrosis

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Abstract

Mesenchymal stem cells (MSCs) are the most widely used in stem cell-based regenerative therapy as they have the unique ability to self-renew and differentiate into many different cell types of a diverse range. The beneficial properties of stem cells therapy come from their tissue repair effect by cellular differentiation and their ability to secrete different trophic factors that exert a favorable impact on the damaged tissue. Also, the therapeutic potential of (MSCs) in the treatment of different diseases is predominantly based on their immunosuppressive properties, and their ability to secrete various trophic factors. This potential has been proven and investigated in various clinical and preclinical studies. For the existing resources of mesenchymal stem cells, there are two main issues should be taken in consideration regarding cell therapy; the first is the source from which the cells are isolated and the second is cell donor type. Liver fibrosis is defined as excess deposition of extracellular matrix (ECM) in response to various liver damages such as viral hepatitis [hepatitis B (HBV) and hepatitis C (HCV)], alcohol consumption, non-alcoholic steatohepatitis (NASH), autoimmune hepatitis, non-alcoholic fatty liver disease (NAFLD), and cholestatic liver diseases

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Introduction

Mesenchymal stem cells (MSCs) are the most widely used in stem cell-based regenerative therapy as they have the unique ability to self-renew and differentiate into many different cell types of a diverse range . The beneficial properties of stem cells therapy come from their tissue repair effect by cellular differentiation and their ability to secrete different trophic factors that exert a favorable impact on the damaged tissue. (1)

Also, the therapeutic potential of (MSCs) in the treatment of different diseases is predominantly based on their immunosuppressive properties, and their ability to secrete various trophic factors. This potential has been proven and investigated in various clinical and preclinical studies. (2)

For the existing resources of mesenchymal stem cells, there are two main issues should be taken in consideration regarding cell therapy; the first is the source from which the cells are isolated and the second is cell donor type. (3)

Sources of MSCs isolation:

MSCs are multipotent stromal cells that can be easily isolated from a variety of tissue sources, including bone marrow, placenta, umbilical cord, amniotic fluid, adipose tissue, dental pulp, breast milk, and synovium. Bone marrow (BM) has became the major source of MSCs which is called bone marrow derived mesenchymal stem cells (BM-MSCs) and they became the preferred cell type in modulating various diseases in veterinary sciences (**4**).

MSCs donor type:

The cell donor can be

- Autologous: the donor is the same cell recipient.
- Allogeneic: the donor is different from the cell recipient.

In recent clinical trials of liver fibrosis and cirrhosis, the trend of MSC therapy seems to have shifted from the administration of autologous cells to allogeneic cells. The choice of autologous therapy is ideal because they ensure major histocompatibility and are unlikely to cause immunological rejection *(5)*. However, autologous therapy still has some potential limitations, and it may be difficult to obtain a sufficient number of healthy active MSCs from patients.

The advantages of using allogeneic compared to autologous MSCs have been fully demonstrated, of which the most notable thing is to obtain cells from healthy donors that have better ability to proliferate to required number in vitro. (6)

Another commonly announced advantage of allogeneic MSCs is their immunomodulatory properties and their low immunogenicity so that they have become a promising approach to treat graftversus-host disease (GVHD) and autoimmune disease. A study introduced the causes of the immunosuppressive properties and low immunogenicity of allogeneic MSCs through changes in the expression of immunogenic markers on the cell surface and changes in the secretion of immunosuppressive molecules. (7)

However, some studies results have showen that allogeneic MSCs may induce a strong immune response in the body which may lead to serious consequences but allogeneic mesenchymal stem cells use still are a promising therapy choice in immunosuppression and tissue repair therapy according to other studies. (8)

Mechanisms of MSC-Based Therapy:

MSCs administration is a promising therapeutic approach as they can promote different tissues regeneration and repair through the migration of cells into target organ, cellular differentiation, paracrine mechanisms and immunoregulation. (9)

MSCs differentiation:

MSCs have plasticity and multidirectional differentiation potential espicially BM-MSCs and adipose tissue-derived mesenchymal stem cells (AT-MSCs). For example, BM-MSCs have invitro and invivo liver differentiation potential to acquire hepatocyte-like cell morphology and hepatocyte-specific markers (including albumin and alpha-fetoprotein). *(10)*

MSCs paracrine mechanisms:

1. Growth factors:

Various cytokines were secreted by stem cells into their conditioned media (CM), and then they played a role in the enhancement of many diseases. The secreted cytokines include different growth factors, anti/pro-inflammatory cytokines and other diverse cytokines.

Growth factors that are secreted by various kinds of MSCs involve vascular endothelial derived growth factor (VEGF), hepatocyte growth factor (HGF), platelet derived growth factor (PDGF), platelet derived endothelial cell growth factor (PDEGF), epidermal growth factor (EGF, insulinlike growth factor I (IGF-I), fibroblast growth factor2/basic fibroblast growth factor (FGF-2/bFGF), heparin binding epidermal growth factor (HEGF), placenta growth factor (PIGF) *(11)* and other diverse growth factors.

VEGF is secreted by almost all types of MSCs, VEGF plays an important role regarding angiogenesis as it helps in regeneration of injured and damaged blood vessels in diseased organs and it may prevent apoptosis thus preventing further tissue damage so, MSCs and their CM achieve an important role especially in ischemic diseases. (12)

Moreover, FGF2 is a more potent angiogenic factor compared to VEGF, with additional effect on proliferation of fibroblasts, preadipocytes, and endothelial, epithelial, and neural stem cells, and on differentiation of neuroepithelial cells into mature neurons and glial cells. *(13)*

Other growth factors that affect angiogenesis are PDGF, TGF- β , HGF, FGF2 and EGF (13). PDGF also contribute in the regeneration of injured tissue as connective tissue, glial tissue, while EGF imitate mesenchymal, glial, and epithelial cells (14).

2. Cytokines:

Various anti-inflammatory cytokines and interleukins (IL) are secreted by MSCs for example IL-10, IL-27, IL-17E, IL-13, IL-12p70, and IL-1 receptor antagonist (IL-1ra). While the secreted proinflammatory cytokines are IL- 6, IL-8, IL-9 and IL-1 β (15).

These cytokines were assessed by multiple studies used different methods to assess various cytokines in the conditioned CM, from the conventional ELISA assays *(12)* to proteomic profiling methods *(16)*.

MSCs immunoregulation:

MSCs exert a wide range of immunomodulative potentials and can regulate the activity of innate and adaptive immune system cells through cell-to-cell contact or secreted factors. However, the underlying mechanisms of MSC-mediated immune regulation have not been fully elucidated so far. *(17)*

Nowadays, there is growing evidence that paracrine factors secreted from MSCs can regulate the immune system by interacting with various immune cells, including macrophages, neutrophils, myeloid-derived suppressor cells, dendritic cells, natural killer (NK) cells, Kupffer cells, T lymphocytes, and B lymphocytes. Indoleamine-2,3-dioxygenase (IDO), and prostaglandin E2 (PGE2) secreted by MSCs are important paracrine factors for MSCs to exert their immunosuppressive effect. (5)

Specifically, IDO are a key mediator of MSC-induced NK cell inhibition. This inhibitory effect is associated with a sharp downregulation of the surface expression of activated NK receptors NKp30, NKp44, and NKG2D. IDO and PGE2 also inhibit the differentiation of Th1 cells, promote the differentiation of Tregs, and increase the migration of CD4 + T cells. *(18)*

Besides, PGE2 is a soluble factor that mediates most of the immunosuppressive of AD-MSCs and BM-MSCs on p-DC maturation and activates T lymphocyte proliferation. Bone marrow dendritic cells (DCs) are also susceptible to this immunosuppressive. The interaction between MSCs and Kupffer cells (KCs) has received little attention, but studies have shown that overexpression of PGE2 in MSCs increases the effect of MSCs on KC reprogramming. The secretion of PGE2 also can enhance the clearance of apoptotic cells (AC) by MSCs. Mechanistically, ACs stimulate MSCs to express COX2, thereby producing more PGE2 that suppresses T-cell responses. NF-xB signaling pathway mediates COX2/PGE2 activation in MSCs. MSCs are also capable of generating an immunoregulatory environment for Treg amplification through a variety of mechanisms. MSCs induce the transformation of fully differentiated Th17 cells into functional Treg cells, thereby regulating the balance of Treg/Th17 cells in the CD4 + T cell population, which is partly attributed to HGF secreted by MSCs. *(19)*

Preliminary observations indicate that the immunomodulatory properties of MSCs derived from different sources are slightly different for example, BM-MSCs and AT-MSCs have simi similar immunomodulatory capabilities, but the difference in cytokine secretion leads to a stronger immunomodulatory effect in AT-MSCs than BM-MSCs. Adipose tissue-derived MSCs (AD-MSCs) express higher levels of IL-6 and transforming growth factor- β (TGF β) than bone marrow-derived MSCs (BM-MSCs) this may be explained by the higher metabolic activity of AD-MSCs. (20)

MSCs in the Treatment of Liver Fibrosis:

Cell-based therapy using MSCs has been proven to be beneficial to alleviate liver fibrosis in some research and clinical studies. As recent technologies and studies have established that MSCs nowadays can be culture-expanded with lesser rejection opportunities after transplantation. Besides, many studies have demonstrated that bone marrow-, umbilical cord-, and adipose tissue-derived mesenchymal stem cells can inhibit liver fibrosis in preclinical animal models, suggesting their potential application in the treatment of liver fibrosis. MSCs have a characteristic plasticity and gigantic differentiation capability. BM-MSCs and Adipose tissue-derived mesenchymal stem cells (AT-MSCs) have a proved liver differentiation potential in vivo and in vitro to acquire hepatocyte-like cell morphology and hepatocyte-specific markers. *(21)*

Therefore, MSCs have the ability to differentiate into hepatocyte-like cells which can be considered as a promising source of liver regeneration. However, hepatic differentiation of MSCs is still a subject of depate for clinical application as they cannot compeletely differentiate into liver cells. Consequently, further research is needed to be done to improve the efficacy and consistency of

differentiation from MSCs to hepatic cells. However, recent studies have established the efficacy of MSCs in treating liver fibrosis such as *Peta et al. (22)* study which reported that human adiposederived MSCs (AD-MSCs) therapy caused a higher antifibrotic factors expression such as IGF-1, IL-10, and HGF and also better capability of protecting hepatocyte injury and apoptosis so the transplanted AD-MSCs are expected to reverse hepatic fibrosis and improve hepatic function.

Moreover, the transplantation of human amnion-derived mesenchymal stem cells (hAMSCs) may provide significant improvement in rat liver fibrosis models by inhibiting the activated Kupffer cells and HSCs. Moreover, a preclinical study shows that the infusion of human amniotic stem cells effectively decreases portal pressure by ameliorating liver microcirculation. These findings suggest that MSCs may serve as new potential approaches to treat liver fibrosis and can be used as a new source of stem cell therapy for liver disease. Furthermore, *Jin et al.*, study demonstrated that tonsil-derived mesenchymal stem cells (T-MSCs) can differentiate into hepatocyte-like cells and ameliorate liver fibrosis via and downregulation of TGF- β /SMAD signalling thus inhibiting the fibrotic process and potentially oppose the liver fibrosis. *(23)*



Fig.1. The potential mechanisms of MSC-based therapy in liver fibrosis. (24).

However, the proliferative capacity and activity of MSCs had established to be affected by culture media and additives (such as glucose level, growth factors, trace elements, lipids, and vitamins) as well as culture conditions and processes, including the oxygen concentration in the incubator, cell dissociating agent, and the density of the inoculated cells. In addition, the regenerative power and the viability of MSCs after transplantation were documented to be significantly decreased by the inflamed microenvironment of the injured organ in vivo so, MSCs pretreatments is a novel method suggested to further strength and enhance the therapeutic effect of the MSCs in different organ damage specifically in liver fibrosis. *(25)*

MSCs pretreatments is accomplished by addition and incubation of MSCs with certain growth factors, cytokines, chemical agents, hypoxic microenvironment and gene modification which will not only protect MSCs against injury but also improve the cellular differentiation, homing capacity, survival, and paracrine effects of MSCs in vitro and in vivo, thus increasing the ability to attenuate liver injury. As, after incubation with some specific growth factors as hepatocyte growth factor (HGF) and basic fibroblast growth factor (bFGF), MSCs had shown high hepatocyte differentiation capacity compared with MSCs without pretreatment methods. *(26)*

Also, a study showed that insulin-like growth factor-I- (IGF-I-) pretreated MSCs are able to induce hepatic macrophages (hM) to transform from profibrotic to a resolutive phenotype, thus driving liver fibrosis amelioration. Besides, the combination of granulocyte colony-stimulating factor (G-CSF) and MSCs had greatly improved the prognosis of patients with advanced liver disease treated with stem cells.

Furthermore, pretreatment of adipose tissue-derived stromal cells (ADSCs) with basic fibroblast growth factor (bFGF) promoted the transdifferentiation of ADSCs into liver lineage cells in vitro, thereby reducing liver fibrosis in mice. Also, in vitro data showed that MSCs pretreated with cytokines have better antifibrosis potential. A new pretreatment method of MSCs also includes gene modification of MSCs which is done by introducing different genes and microRNAs into MSCs through viral or nonviral vectors to improve their differentiation, immune regulation, homing ability, and other repair-related abilities. Hepatocyte growth factor (HGF) is a potent mitogen for mature hepatocytes, which has been shown to play critical roles in liver regeneration and has been applied in gene therapy in cirrhosis and achieved a better therapeutic effect. *(27)*

Hence, HGF were broadly used to modify MSCs to improve the therapeutic effects on liver fibrosis. A study showed that cell therapy with genetically engineered BM-MSCs, which were overexpressed HGF mediated by adenovirus, significantly promotes liver function and attenuates liver fibrosis than treatment with MSCs alone. A study also showed that transplantation of human HGF-overexpressing human umbilical cord blood-derived MSCs (hHGF-HUCB-MSCs) in CCL4-induced rat liver fibrosis model has higher liver function improvement and lower collagen fiber deposition than treatment with unmodified HUCB-MSCs. Moreover, erythropoietin (EPO), FGF4, FGF21, IGF-1and IL-10 modified MSCs also showed better therapeutic effect in the treatment of liver fibrosis. *(28)*

In addition, gene-modified MSCs can also enhance the immunomodulation of MSCs, thus increasing the therapeutic effect of MSCs. For example, MSCs overexpressing IL-35 have higher immunosuppressive capabilities. IL-35-MSCs induce CD4 + T cells to produce IL-10, but have no effect on IFN- γ . Therefore, modifying some specific genes is a potential new strategy for treating liver fibrosis. (28)

MSCs Derived CM:

Various studies were done on stem cell-derived secreted factors established that MSCs secreted factors alone without the stem cell itself may cause tissue repair in various conditions that involved tissue/organ damage. These secreted factors are referred to as secretome, microvesicles, or exosome and they are found in the medium where the MSCs were cultured in; this medium is called MSCs derived conditioned medium (MSCs-CM). The conditioned medium can be harvested from various kinds of cells . Moreover, there are various methods to get the conditioned medium, which affect the growth factor types and levels that were harvested by these methods. *(29)*.

Some of the various studies that used MSCs-CM checked the growth factor levels by using different methods to assess various cytokines in the conditioned media (CM), from the conventional enzyme- linked immunosorbent assay (ELISA) to proteomic profiling methods. Very limited data are available regarding the use of CM in regenerative therapy like hair follicle regeneration and wound healing (4).

MSC-CM as a therapy:

Production method of the MSCs-CM needs to be standardized regarding the number of cells that produce the CM, conditions of culture medium and CM processing to use it as a therapy for different human diseases. In addition, the volume and mode of delivery are also important as various studies used a different numbers and type of cells and various doses of CM, it is also important to know the number of cells that yielded the CM before their application for human studies. (*30*).

MSCs-CM administration is now considered a hopeful therapeutic method as they contains all MSCs-secreted products as secretomes, microvesicles and exosomes helping in different tissues regeneration and repair.

• Culture media preparation:

The cultured BM-MSCs were allowed to grow for 10-14 days to reach 80-90% confluence which is characterized by their fusiform shape detected by inverted image microscope and their adhesiveness to the bottom of the flask. Then, the media was changed and the cells were cultured in serum free DMEM for 24 hours then the CM was collected in a sterile tube and used as a therapeutic method. *(31)*.

• Culture duration:

Production of CM varies in culture duration from sixteen hours to five days . In case complete medium was used, short culture duration might leave certain serum derived growth factors that wasn't consumed by the cells and might increase the growth factor level, this can be seen in a study, which showed that medium without cell contained a TGF- b1 level of $2.49 \pm 2.39 \text{ pg/mL}$. (32)

• Culturing conditions:

Some studies produce CM from cell culture in normoxia (O2 level 20-21%), however oxygen deprivation (hypoxia O2 level 0.5%, 1%, 1.5%, and 2%) is better condition as different studies have reported that most growth factors were upregulated in hypoxic condition, for example, vascular endothelial derived growth factor (VEGF) hepatocyte growth factor (HGF), platelet derived growth factor (PDGF), placenta growth factor (PIGF), and insulin-like growth factor II (IGF-II) *(11)*, except for epidermal growth factor (EGF) that was downregulated *(33)*.

Translation of CM:

Therapeutic effects of MSCs-CM:

MSCs-CM contains all cell-secreted products has demonstrated various therapeutic benefits for different diseases such as acute liver injury/failure, stroke, myocardial infarction, neurodegenerative diseases, alopecia, acute and chronic wounds, lung injury, periodontal tissues injury, male infertility, soft tissue and bone defects. MSC-secreted extracellular vesicles that carry regulatory noncoding RNAs were also used as therapeutic agents to stimulate tissue regeneration. Thus, MSCs secretome is suggested as a novel cell-free therapeutic product that can be equivalent to the beneficial effects of MSCs therapy and has various advantages in overcoming the limitations and risks associated with cell-based therapy. *(34)*

Concerning about the beneficial effects of MSCs-CM therapy, there are several clinical trials including the use of MSC-CM for hair follicle regeneration (32), fractional carbon dioxide resurfacing wound healing (11) as well as for inflammatory arthritis), and multiple sclerosis. (35)

Furthermore, concerning angiogenesis effect of MSCs-CM based therapy, MSCs-CM was found to be able to cure various diseases and have more impact on diseases with ischemia as studies that analyzed VEGF level in culture medium have reported that most stem cells secrete VEGF (12) that is important in regeneration of injured tissues and prevention of apoptosis thus preventing further damage. Other than VEGF, other growth factors also were found to be present in CM as FGF2, EGF, HGF, SDF-1, PDGF and PIGF that may play a role in angiogenesis. In addition, various cytokines such as interleukins, chemokines (13), monocyte chemotactic protein (MCP-1) IL-8 (29). leptin and angiogenin also may play a role in angiogenesis. (36)

Advantages and disadvantages of MSCs-CM:

The use of CM for therapy is very appealing and may be successful in the near future, as studies on the use of CM for various diseases are accumulating. The use of secretome containing CM has several advantages over MSCs, as CM can be manufactured, freezed, packaged and transported more easily. Moreover, as it is devoid of cells; there is no need to match the donor and the recipient to avoid rejection problems. Therefore, MSCs-CM have a promising prospect to be produced as a therapy for regenerative medicine. According MSCs therapy, several safety factors related to MSCs transplantation still a topic of importance because of the potential risk of immune reactions and cancer development. Furthermore, insufficient viability of transplanted cells and poor engraftment restrict their therapeutic efficacy. *(37)*

However, significant variability of approaches to MSC-CM bioprocessing has a serious impact on experimental outcomes (16). Particularly, the need for disease-specific identity and potency testing due to undefined mechanisms of action of MSC secretome makes development of this class of biopharmaceuticals more complicated, expensive and precarious. However compared to MSCs, MSCs can survive for a long period but MSCs-CM contain cytokines and growth factors with shorter half-lives so the treatment with MSCs-CM needs to be given more frequently which is a disadvantage for the receiving patients. Additionally, the composition of MSCs-CM is intensely influenced by donor variability, tissue of origin and this should be considered during CM preparation. (13)

Liver fibrosis is defined as excess deposition of extracellular matrix (ECM) in response to various liver damages such as viral hepatitis [hepatitis B (HBV) and hepatitis C (HCV)], alcohol consumption, non-alcoholic steatohepatitis (NASH), autoimmune hepatitis, non-alcoholic fatty liver disease (NAFLD), and cholestatic liver diseases. The mutual effect of these factors on the liver is generating a chronic inflammation state resulting in disturbed hepatic architecture, excessive scarring and abnormal healing response at which different cell types and mediators participate to encapsulate injury. *(38)*

In response to chronic liver injuries, various cell types as hepatic stellate cells (HSCs) get triggered and turn into activated HSCs (aHSCs) which considered as the key cell type driving liver fibrosis (29). HSCs are quiescent in normal livers (qHSCs) but in damaged livers they transdifferentiate into myofibroblastic cells that participate in synthesis and reorganization of connective tissue and produce different matrix proteins in particular collagen I and II. The main source of extracellular matrix (ECM) production are HSCs undergoing myofibroblastic transition. Myofibroblastic

differentiation and matrix accumulation by aHSC is heavily triggered by profibrogenic mediators such as transforming growth factor β (TGF- β) and the β -isoform of platelet-derived growth factor (PDGF) leading to fibrous scar formation, hepatocyte loss and the deregulation of the normal functions of the liver, ultimately resulting in liver failure. *(39)*

Liver fibrosis in its early stages is a reversible process, unless it becomes progressive and propagates to hepatic cirrhosis. The removal of the fibrotic response-causing agent aids in the regression of fibrosis as long as the liver is not at the stage of advanced cirrhosis. Advanced stages of liver fibrosis eventually proceed to cirrhosis and attribute to infiltration of liver by inflammatory cells and the apoptosis of hepatocytes. The condition could deteriorate further more and hepatocellular carcinoma (HCC) may develop as up to 90% of HCC develops on the basis of liver fibrosis or cirrhosis. (40)

Pathogenesis of liver fibrosis

Liver fibrosis is a serious health problem with numerous complications such as liver cirrhosis and hepatocellular carcinoma (HCC). Fibrogenesis is initiated by myofibroblast activation and proliferation because activated myofibroblasts are the major source of ECM in the injured liver. Although activated hepatic stellate cells (aHSCs) are the major source of myofibroblasts in the fibrotic liver, they are not the only precursors. Endogenous portal fibroblasts, fibrocytes, bone marrow-derived cells, and liver parenchymal cell-derived myofibroblasts that undergo epithelial-mesenchymal transition (EMT) give rise to a significant percent of myofibroblasts in the fibrotic liver. Different cell types activate myofibroblasts depending on the etiology of liver fibrosis. A previous study demonstrated that aHSCs are the source of myofibroblasts in a carbon tetrachloride (CCl4)-induced liver fibrosis model, whereas portal fibroblasts give rise to myofibroblasts in the cholestatic liver. Bone marrow-derived cells represent a substantial fraction of the total fibrogenic population in a more chronic injury. **(41)**

In the quiescent state, HSCs are known as quiescent HSCs (qHSCs), and they are responsible for the storage of vitamin A in the liver. As a result of liver injury, qHSCs are activated by inflammatory mediators into aHSCs, which in turn differentiate into myofibroblasts. In this way, tissue remodeling is initiated in the liver by the secretion of ECM proteins and matrix metalloproteinases (MMPs) by aHSCs.Besides, studies to clarify the signaling molecules that contribute to the activated HSC have identified one signaling molecule called CCN2, a profibrotic factor that is produced in fibrosing liver tissue. CCN2, a cysteine-rich matricellular protein, interacts with integrins, low-density lipoprotein receptor-related proteins, and heparan sulfate proteoglycan coreceptors, thus stimulating adhesion, migration, proliferation, survival, and differentiation of HSCs. CCN2 exhibits strong profibrogenic properties. Overexpression of CCN2 promotes ECM deposition and development of fibrotic lesions. Hepatic levels of CCN2 correlate with the severity of liver disease in patients with liver fibrosis. Additionally, overexpression of CCN2 mediates TGF-\beta1-dependent fibrotic pathways in HSCs, and TGF-\beta1 mRNA transported by injured epithelial-derived exosomes results in a rapid initiation of activation of myofibroblasts. Similarly, stress activators such as Brefeldin leads to increase in the deposition of collagen along with increased Smad-3 signalling and expression in hepatic stellate cells thus resulting in subsequent hepatic fibrosis. (42)

Mechanisms of liver fibrosis

Different factors for example toxins, hepatitis, steatohepatitis, and autoimmune disorders, promote the activation of HSCs, which in turn acquire a myofibroblast-like phenotype. HSC activation comprises two major phases named initiation and perpetuation, then these processes are followed by a final resolution phase in case the injury cause still exists. Soon after the injury, the initiation phase begins to generate a response against the factors that cause injury at the gene expression level, resulting in different phenotypic changes. Early activation is driven by the presence of damaged hepatocyte products, causing HSCs priming, activation and preparation for fibrotic events.Continuous stimuli from primed cells after their activation will result in progression to perpetuation phase. Perpetuation phase cell behavior is characterized by proliferation, fibrogenesis, contractility, matrix degradation, chemotaxis, retinoid loss and cytokine release. Overall, these changes contribute to the accumulation of ECM and fibrotic changes. *(43)*

Certain growth factors also stimulate HSC proliferation as transforming growth factor (TGF- β) and epidermal growth factor (EGF). Normally, TGF- β 1 is found inactive but following its activation, it initiates *SMAD* signaling pathway resulting in collagen production. Moreover, TGF- β 1 promotes the trans-differentiation of qHSCs into myofibroblasts that secrete ECM. Besides, hepatic angiogenesis is initiated by another growth factor called vascular endothelial growth factor (VEGF). Also, platelet-derived growth factor (PDGF) has a significant role in the activation of HSCs, it encourages cellular proliferation by recognition and dimerization of its receptors thus promoting receptor autophosphorylation and activation of Ras-dependant protein kinase pathway that increases intracellular calcium and results in protein kinase C activation. Overall, these growth factors promote the fibrotic progression and the remodeling of ECM resulting in collagen formation. In the normal liver, collagen IV and VI are present in the space of Disse. However, during fibrogenesis, they are replaced by collagens I and II and fibronectin. (44)

In addition, HSCs are capable of expressing different chemokine receptors (CXCR) and chemokine receptor ligands (CCL). Chemokines are a class of small chemotactic molecules that regulate inflammation and the migration ability of fibrogenic cells to the injury site thus increasing the number of cells and inflammation at the site of injury. Both profibrogenic and antifibrogenic effects occur on interaction between different chemokine receptors and its ligands. The inflammatory pathways play a significant role in liver fibrogenesis. There is a positive feedback loop between inflammatory and fibrogenic cells, which in turn results in amplified fibrosis. The activation of HSCs is promoted with many other cell types such as Kupffer cells, natural killer (NK) cells, T cells, macrophages, dendritic cells, and endothelial cells. The increased Kupffer cell activity predominantly upsurges the expression of nuclear factor-kappa β , which in turn promotes pro-inflammatory cytokine secretion. Moreover, oxidative stress and apoptotic cells are capable of inducing immune response thus increasing collagen production, HSC migration and cause progressive liver injury. These events result in vascular disorganization, hypoxia and initiation of angiogenesis. A hypoxic environment induces VEGF and PDGF cytokine activities that promote both fibrogenic and angiogenic responses. Activated HSCs by hypoxia initiate interactions with PDGF and VEGF signaling, which play an important role in angiogenesis. (45)

Reversibility of liver fibrosis

The reversibility of liver fibrosis was a controversial issue for a long time because some of the earlier studies argued that liver fibrosis is irreversible. However, more recent studies have supported the idea that it is a reversible process if the injury-causing stimulus is withdrawn and

demonstrated this argument in both experimental liver fibrosis models and clinical samples of a cirrhotic human liver.With the withdrawal of the causative agent, a cascade of events occurs to initiate the reversion of the fibrotic response for example, decrease in cytokine levels, increase in the collagenase activity resulting in diminishing of fibrous scars, myofibroblastic fading through senescence and apoptosis. Moreover, the loss of myofibroblasts results in a decrease in tissue inhibitors of metalloproteinase (TIMP) levels and an increase in matrix metalloproteinase (MMP) activity, thus degrading ECM. MMPs are calcium-dependent enzymes that specifically degrade collagens and non-collagenous ECM substrates. HSCs secrete different basement membrane proteases MMP-2, MMP-9, MMP-3, MMP-13 and interstitial collagenase. The inactivation of these proteases by binding to TIMPs is also emerging as an important locus of control because the sustained production of these proteins during liver injury could inhibit the activity of interstitial collagenases, leading to reduced degradation of the accumulation of ECM proteins. In addition, TIMP-1 is anti-apoptotic for HSCs, which may result in an increased number of aHSCs. Although, the loss of myofibroblasts is not the only component of liver fibrosis regression but also, macrophages control have a significant role in the progression and resolution of liver fibrosis. As during the progression of liver fibrosis, macrophages augment fibrogenesis by producing cytokines and chemokines that induce HSC transition into myofibroblasts. (46)

Antifibrotic therpies

Thanks to continued experimental advances in the past years, new promising and exciting therapeutic approaches can be developed. One of the active research areas to develop new therapy is toward targeting fibrogenic events in the liver. TGF- β is a well-known molecule that occurs in fibrotic events in all organs. However, its systemic inhibition may increase overall inflammation. Thus, targeting certain steps in the activation of TGF- β pathway by different miRNAs as miRNA-24 which targets $TGF-\beta/SMAD$ signaling may be helpful to decrease the fibrotic response in the liver. Also, connective tissue growth factors and integrins are good candidates for targeting the $TGF-\beta$ signaling pathway because they play significant roles in TGF- β release and activation. Reducing redox injury by using numerous antioxidants is another alternative for antifibrotic therapy. But unfortunately, owing to differences between animals and humans testing the effect of antioxidants on liver fibrosis in humans is more complex than predicted and more clinical trials need to be conducted to insure and assess their effects. Another possible area to develop new therapies against fibrosis is in the targeting macrophage recruitment which may be a useful approach in rodents because macrophages play a predominant role in fibrogenesis and its regression. As liver fibrosis is a dynamic process, targeting one pathway only in this process may not be enough to induce its reversal. Combination therapies that target the chief components that underlie liver fibrosis such as ECM proteins and certain cell types are very promising. (47)

Overall, combination approaches for antifibrotic therapies are very hopeful nowadays but their harmful, toxic, off-target side effects should be demonstrated in future studies, like in many other therapeutic approaches for different diseases. *(18)*

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