The role of SEVs in the promoting and treating liver fibrosis-MASP-1-enriched SEVs promote liver fibrosis while MSCs' SEVs treating it

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Abstract

Liver fibrosis is a chronic severe liver disease. One prominent feature of liver fibrosis is the activated Hepatic stellate cell. Across all the therapeutic tools for liver fibrosis, Mesenchymal stromal cells are widely studied and used. The MSCs treat liver fibrosis by secreting cargo. One is an extracellular vesicle called an exosome containing proteins and other secrets. As the critical role of exosomes in the cell's communication and interchange of substances, more research is reported. Five types of exosomes from different sources that each have different roles in either promoting or inhibiting liver fibrosis are reviewed.

Keywords: Liver fibrosis, HSCs, exosomes, MSCs.

1. Introduction

Exosomes also known as SEVs are one type of small extracellular vesicles which secret by multivesicular endosome pathway. Its diameter is the smallest compared with micro vesicles and apoptotic bodies around 30-150nm [1,2]. Although it is small, it contains plenty's of important chemical substances and biomolecules like transcription factor and proteins. What's more, the exosomes have lipid bilayer which have substances that are useful in scientific fields like CD63 and other tetraspanins. Its unique structure lays the foundation for its important function on cell communication and interchange of material. Since James Rothman own the Nobel Prize in Physiology or Medicine for his work on the machinery regulating vesicle traffic, a major transport system in our cells, more and more scientists started to work on the research related to exosomes. More and more scientists focused on the important role of exosomes in medic field. As the COVID-16 spread widely these years, many research paper of coronavirus has been published. Oksana Kolesova and her teams finds the evidence for the induction of liver fibrosis by multiple factors during acute COVID-19 [3]. The liver fibrosis has long been considered a serious global health risk, and it is chronic disease and could be caused by abnormal liver microenvironment and the continuous activation of hepatic stellate cells (HSCs). For more effective and convenience treatment of liver fibrosis, scientists put effort on the detailed pathogenesis. There are lots of chemical particles and biomolecules including exosomes are involved in the pathogenesis, plenty of research and experiments has been taken to examine the role of each substance. The least paper points out the important role of exosomes on promoting liver fibrosis which draws the attention of scientists to the research on exosomes again. To review the research process and give the prospect of the future research on the exosome and its role in the liver fibrosis or other diseases, the key papers about this topic would be represented and discussed.

2. Liver fibrosis

Liver diseases are serious medic problem and the biggest risk for humans' life, almost 2 million deaths per year could be caused because of them [4]. The liver fibrosis is one of the liver diseases which could be caused by viral or metabolic chronic liver diseases. During the process of liver fibrosis which is the consequence of the liver repairing to maintain a balance between fibrogenesis and fibro lysis, the parenchymal cells will decrease and their ability to regenerated will be weaken [5]. And the transdifferentiate of Hepatic stellate cells (HSCs) into collagen-producing myofibroblasts will be activated because the damage of hepatocytes and the infiltration of immune cells caused by the pathogenic factor [6,7]. The myofibroblasts are very important because the damaged tissues need the extracellular matrix (ECM) to replace them and the main producer of the components of ECM is myofibroblasts [8-10]. The excessive deposition of ECM which is the effect of liver fibrosis will destroy the physiological structure of liver. Without the effective medical care, the unbalanced liver microenvironment and fibrogenesis and fibro lysis will lead to liver cirrhosis which is reported as one of the most common deaths in the world [11].

2.1 *The therapeutic tool-MSCs*

There are lots of research which focus on the therapeutic

tool for liver fibrosis, one of them is called Mesenchymal stromal cells (MSCs). It can not only be easily cultured, but they could also be collected easily from bone marrow and some tissues like the umbilical cord tissue and adipose tissue. Although they could still differentiation, they secret cytokines, chemokines, growth factors, and exosomes to do their usage instead of just replace the damaged cells or tissues [12,13]. It has been proposed that the SEVs from the MSCs mediated the ability of MSCs to regenerate and they have similar therapeutic potential because they are secreted from MSCs [14,15]. In 2014, Feng et al. found the MSC-derived SEVs which contains miRNA-22 reduced infarct size and cardiac fibrosis by inhibiting apoptosis in mice with myocardial infarction. This result points out the great role of SEVs in the MSCs-mediated tissue repair and regeneration [16,17]. The tonsils have been considered as a novel promising source of MSCs, and it has been also reported that the tonsils-derived MSCs (T-MSCs) which collected from tonsil tissues could treat liver fibrosis by differentiating hepatocyte-like cells or promoting autophagic flux [18,19]. The mechanism of T-MSCs inhibiting liver fibrosis and whether the SEVs from T-MSCs influence the disease or not is still unclear until Jieun Kim et al. took an experiment to figure out the role of SEVs from T-MSCs in liver fibrosis.

2.1.1 *The role of T-MSCs-dervied SEVs*

They firstly found the T-MSCs could promote inactivation of HSCs by comparing the RNA levels of HSC activation markers like transforming growth factor-β (TGF-β) and connective tissue growth factor (CTGF), the HSC inactivation marker glial fibrillary acidic protein (GFAP) was also compared in two human activated HSC line LX2 cultures, T-MSC-CM group and the control group. Then they use the human activated primary hepatic stellate cells (PHSCs) treated with T-MSCs-derived SEVs at differentiation concentration to assess the effect of SEVs on activated HSCs. As the figure 1 shows, the culture which contains T-MSCs all shows that the levels of the activation makers like TGF-β, CTGF and Vimentin are decreased to different degree [17]. Further experiment showed the SEVs could alleviated liver damage. As miRNA is one of the containers of SEVs which could be used to regulate the expression of target gene on the target cell, four types of miRNAs, miR-185-5p, miR-125b-5p, miR-486-5p, and miR-130a-3p which are abundantly expressed in T-MSCs [20]. The level of miR-486-5p is much higher than the other three miRNA especially in the T-MSCs-derived PHSCs, and the activation makers are also decreased, thus the target of miR-486-5p which is smoothened (SMO), a receptor for the Hedgehog signaling (Hh) pathway is curtained and confirmed that the miRNA could block the pathway and inactivating HSCs.

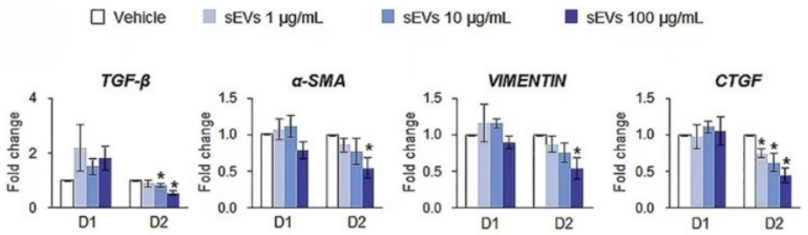

Figure 1. qRT-PCR analysis for TGF-β, αSMA, COL 1α1, VIMENTIN, CTGF, TIMP1, and GFAP in human pHSCs cultured alone (pHSC alone) or co-cultured with T-MSCs (pHSC with T-MSC) for 1day (D1) or 2 days(D2) [17].

2.1.2 *The role of hBM-MSCs-dervied SEVs*

Previous research points out the liver fibrosis could be moderated by downregulating the Wnt signaling pathway via blocking the bone morrow-derived MSCs (BM-derived MSC) [21]. And Rong with his team found out the usage of hBM-MSCs-derived SEVs. The model of 8-week CCL4-induced mice which could simulate the liver injury was used in this experiment to detect the liver function and some inflammatory cytokines after 4 weeks hBM-MSCs-derived SEVs vivo administered. They also compared the result with the hBM-MSCs injected mice and found out the SEVs have greater therapeutic effect against liver fibrosis. In addition, they found the mechanism of the SEVs alleviating the liver fibrosis which is they could inhibit the components of Wnt/β-catenin pathway like Wnt3a, β-catenin, WISP, α-SMA, and Collagen I to be expressed [22].

2.2 *Macrophage*

Macrophage is another type of cell therapy for inducing liver fibrosis instead of MSCs. It is an important factor in the process of regulate and resolute the liver fibrosis by the exogenous colony stimulating factor-1 (CSF-1) which could inactivate the macrophage infiltration and improve its function in renal or cardiac fibrosis [23,24]. Instead of MSC and macrophage monotherapy, Watanabe et al. use the bone marrow-derived macrophages and MSCs found the synergia of ameliorating liver fibrosis, but the MSCs do not head to the damaged area of liver but stuck in the

lung while the macrophage migrated to the liver [23]. The MSCs do not react directly but use some cargos which contained in the MSCs react with the macrophage in this synergic relationship, and it could be MSCs-derived SEVs.

2.2.1 *The role of SEVs in MSC-macrophage communication*

The MSCs could robust anti-inflammatory during the process of strong inflammatory and interferon γ(IFN-γ) is considered as the key factor which could change the character of MSCs [25]. Takeuchi et al. collected the SEVs which following pre-conditioning with IFN-γ and added to bone marrow-derived macrophages (BMMs), compared with the normal SEVs found the IFN-γ-derived SEVs have a greater impact on decreasing the levels of pro-inflammatory macrophage factors and increasing the levels of anti-inflammatory macrophage factors, that showed that adipose tissue-derived MSCs (AD-MSC-γ-SEVs) effectively induce anti-inflammatory macrophage responses in vitro, as the SEVs contain anti-inflammatory macrophage inducible proteins like annexin-A1 which also showed the usage of IFN-γ induced proteins in the communication between macrophage and MSCs . And the further experiment's result on the macrophage motility and phagocytic activity showed the promotion role of AD-MSC-SEVs and AD-MSC-γ-SEVs on the macrophage motility and phagocytic activity. In the vivo experiment by using the CCl4-induced mice, the IFN-γ precondition could increase the number of anti-inflammatory macrophages and regulatory T cells so that the liver fibrosis could be inhibited. And the role of MSC-derived SEVs that could cause accumulation of CX3CR1+ macrophages in damaged areas was also found through investigating the behavior of macrophages with SEVs in CX3CR1-EGFP mouse as the CX3CR1+ cells are similar with the scar-associated macrophages (SAM) which facilitate the resolution of murine hepatic fibrosis [26,27].

2.3 *The role of SEVs in activating liver fibrosis*

During the process of liver fibrosis, many SEVs are secreted and some of them contains self-RNA, the self-non-coding RNAs are considered as the activator of the Toll-like receptor 3(TLR 3), which are also called CD283 that can induce the production of type I interferons. Seo et al. compared the TLR3 knock out CCL4 injected mice with the wild type CCL4 injected mice, found the role for HSCs in interleukin-17A (IL-17A) production by γδ T cells. And the vitro injection with the SEVs from the CCL4 treated hepatocytes showed the SEVs mediated the TLR 3 produce I interferons especially the IL-17A. As the further reciprocal bone marrow transplantation between WT and TLR3 KO mice showed the positive correlation between liver fibrosis and IL-17A, they have verified the SEVs from the HSCs mediated the TLR3 promotion of liver fibrosis by enhanced the γδ T cells producing more IL-17A [28].

The autophagic-lysosomal pathway is very important in homeostasis, the HSCs could be activated by the lipid droplets which produced by autophagy, and the inhibition of autophagic like 3-methyladenine could inhibit the autophagy so that the activation of HSCs would be weaken which will ameliorate liver fibrosis [29,30]. In addition, the intercellular biodegradation system controls the multivesicular endosome pathway and the multivesicular body (MVB)/EV route, the EV secretory activity is closely related to it, the activated system would reduce MVBs-membrane fusion though the autophagic-lysosomal pathway so that less EVs would be secreted [31]. When weaken, by contrast, more EVs would be secreted. However, how the autophagy-lysosomal pathway affects the hepatocytes-derived SEVs secretory activity on the activation of HSC and fibrogenesis was not reported in past papers.

Liu et al. found the β-arrestin1(ARRB1), a scaffold for intracellular signaling networks that could regulate autophagic flux, is participated in the liver fibrosis and correlated with suppressed hepatocyte autophagy. Their experiment's result on the ARRB1-deficiency mice also support the finding. They also found a lysosomal marker located in the lysosomal membrane named LAMP-1 decreased through LAMP1 ubiquitination in the ARRB1 overexpression hepatocytes. The decreased LAMP1 also weaken the interaction between LAMP1 and LC3B that the autophagosome-lysosome fusion was blocked and result in the MVB degradation which will lead to more SEVs secreted. Further experiment pointed out, as the level of Rab27 A mRNA which is responsible for MVB trafficking and small EV secretion was also increased in the ARRB1 overexpression hepatocytes, the ARRB1 bind to the Rab27 A receptor that the expression is increased so that the more SEVs secreted [32]. After analyzing the proteins in the SEVs, Mannan-binding lectin serine protease 1 (MASP-1) has 17 times decreased in ARRB1-KO mice compared with wildtype. They examined if the ARRB1 target to MASP-1 though a dual-luciferase reporter system, and they found ARRB1 could bind to MASP-1 promoter so that increase the production and secretion of hepatocyte-derived MASP1-enriched SEVs to activate HSCs in liver fibrosis. In addition, with further experiment, they found the liver fibrosis is promoted due to the MASP-1 promote the activation of HSCs through p38 MAPK/ATF2 signaling [33].

3. Conclusions

Five types of exosomes are reviewed in this report, including its role in the liver fibrosis which derived from tonsils and human bone marrow. Bone marrow has been widely used as the source of MSCs, but the limitation like the low yield and the great pain of taking bone marrow from human make it hard to take into clinical test [34]. The tonsils could be easily collected, and it has similar function and structure, which make it a great source of MSCs [18]. Thus, the experiment on the tonsils could be more appliable. The role of exosome in the communication between macrophage and MSCs is also talked, the synergia of ameliorating liver fibrosis by using the macrophage with MSCs could be a new idea of clinical when treating liver fibrosis. But the experiments did not find the usage of cargo in the IFN-γ pre-conditioned SEVs and the effect of IFN-γ on SEVs structure and functions, thus further research should be focus more on the IFN-γ pre-conditioned SEVs. In addition, a more convenience and efficient of filtrate IFN-γ pre-conditioned SEVs should be set up. The last two types of exosomes focus on the promotion role in the liver fibrosis. These experiments show the new novel therapeutic targets like TLR3 and MASP-1, and both show the importance of autophagic. But there are few research related to the γδ T cells and the ARRB1, more studies should be taken on them. As one of the most serious diseases in the world, there should be more research on the liver fibrosis, especially the exosomes. However, the high equipment of screening exosomes and hard to be approach to clinical test are two hard problems. So, better way to separate exosomes and more tolerated way to collect exosomes should be found out.

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