Frontiers in Bioscience Landmark

Front. Biosci. (Landmark Ed) **2022**; 27(3): 104
https://doi.org/10.31083/j.fbl2703104

Original Research

Fluid Shear Stress-Induced Exosomes from Liver Cancer Cells Promote Activation of Cancer-Associated Fibroblasts via IGF2-PI3K Axis

Tang Feng[1], Fei Fang[1], Chunhe Zhang[1], Tiantian Li[1], Jia He[1], Yang Shen[1], Hongchi Yu[1,*], Xiaoheng Liu[1,*]

[1]Institute of Biomedical Engineering, West China School of Basic Medical Sciences & Forensic Medicine, Sichuan University, 610041 Chengdu, Sichuan, China

*Correspondence: liuxiaohg@scu.edu.cn (Xiaoheng Liu); yuhongchi@scu.edu.cn (Hongchi Yu)

Academic Editors: Antonio Barbieri and Francesca Bruzzese

Submitted: 15 January 2022 Revised: 20 February 2022 Accepted: 2 March 2022 Published: 17 March 2022

Abstract

Background: Cancer-associated fibroblasts (CAFs) are of considerable importance in tumor progression by interacting with the tumor microenvironment. However, the hidden mechanism explaining how tumor cells interact with CAFs in the tumor mechanical microenvironment remains largely unknown. **Methods**: We highlighted exosomes as the mediator modulating the interaction between liver cancer cells and CAFs under mechanical conditions. The normal hepatic stellate cells LX2 were exposed to the medium or exosomes from the HepG2 cells with or without fluid shear stress subjection, and the CAFs activation markers were checked. To further explore the potential role of PI3K, which is active in liver fibrosis, the PI3K inhibitor was used. **Results**: The specific markers of CAFs, FAP, and α-SMA, increased in LX2 with subjection to the fluid shear stress-induced exosomes from HepG2 cells. In turn, the enriched IGF2 in the exosomes activated the IGF2-PI3K signaling pathway in LX2 cells. **Conclusions**: These findings reveal that fluid shear stress-induced liver cancer cells possess a stronger capacity to convert normal fibroblasts to CAFs than statically cultured liver cancer cells, and tumor-derived exosomes mediated the intercellular cross-talk between liver cancer cells and fibroblasts.

Keywords: fluid shear stress; cancer-associated fibroblasts; exosome; IGF2; cell-cell communication

1. Introduction

The tumor microenvironment (TME) is composed of the surrounding fibroblasts, immune cells, cytokines, chemokines, as well as the extracellular matrix (ECM) [1–3]. Cancer-associated fibroblasts (CAFs), the amplest fibroblasts in the cancer stroma, is a continuously activated subpopulation of fibroblasts, playing a crucial role in promoting tumor progression [4,5]. CAFs exhibit a high degree of heterogeneity because of the diversity of sources and expressed different specific markers for identification. In the liver and pancreas, the static stellate cells activation is the main source of CAFs [6]. Thereinto, α-SMA, FAP, and PDGFR-β are well-known markers for identifying CAFs [7,8]. It has been found that cytokines, inflammation, hypoxia, autophagy cause the activation of CAFs [9–11]. On the other hand, increasing evidence showed that the cancer cells are the most crucial factor for educating normal fibroblasts (NFs) to CAFs [12]; those research suggested that there was cellular cross-talk between CAFs and cancer cells [13,14]; however, the potential candidate activating fibroblasts is far from clear.

There are various biological and physical abnormalities in the TME. Among these, biomechanical properties alterations of the TME are a new hallmark of cancer [15,16]. Here, in the tumor biomechanical environment, due to the recruitment of leaky blood vessels and deficiency of functioning lymphatic vessels, there's a rise in the interstitial fluid pressure (IFP), causing an abnormal interstitial flow which induces elevated fluid shear stress (FSS) [15]. 0.1–2 dyn/cm^2 FSS has been identified to enhance cancer cells migration and invasion, as well as tumor-associated angiogenesis [17]. Our previous studies had also validated the catalytic effect of FSS on the migration of liver cancer cells [18,19]. On the other hand, the potential contributing role of cancer cells subjecting to FSS in the interaction with CAFs, has not been deeply studied.

Exosomes, a kind of extracellular vehicle with a diameter between 30 nm and 160 nm, are widely involved in cell-cell communication [20]. Exosomes secreted from cancer cells could be absorbed by fibroblasts and reshape the biological functions of the recipient cells, finally resulting in the CAFs activation [21,22]. Therefore, the altered bioactive components, which are contained in the exosomes released from FSS-induced liver cancer cells, may activate CAFs.

IGF2, playing a critical role in regulating cell proliferation and migration in solid tumors, is found to be upregulated in a variety of human malignancies, including liver cancer, and is associated with poor prognosis [23]. In liver cancer, though the loss of imprinting, loss of heterozygosity, or reactivation of IGF2 transcription [24] could partially explain the upregulation of IGF2 in cancer, the detailed functions and mechanisms about IGF2 release in the TME still remain unknown. Whether the aberrant expres-

sion of IGF2 in exosomes derived from FSS-induced liver cancer cells could mediate the interaction of tumor cells and CAFs needs to be elucidated.

Here, we hypothesize that the medium from liver cancer cells with fluid shear stress subjection is more capable of activating fibroblasts through the bioactive substance in exosomes. Therefore, we investigated the activation of normal hepatic stellate cells LX2 treated with the medium or exosomes from liver cancer HepG2 cells in the absence or the presence of fluid shear stress. Furthermore, we explored the molecular process of IGF2 in exomes activating the recipient fibroblasts.

2. Materials and Methods

2.1 Cell Culture

The human liver cancer cell lines HepG2, QGY-7703, and hepatic stellate cells LX2 (Institute of Biochemistry and Cell Biology, Shanghai, China) were cultured in an incubator with 5% CO_2 at 37 °C. RPMI-1640 (Gibco, Grand Island, USA) was used to culture HepG2 and QGY-7703 cell lines. The high glucose DMEM (Gibco, Grand Island, USA) was used to culture LX2 cell lines. The culture medium was supplemented with 10% fetal bovine serum (Gibco, Grand Island, USA) and 1% penicillin- streptomycin (Gibco, Grand Island, USA).

2.2 Fluid Shear Stress Loading

The fluid shear stress loading system and HepG2 cell loading conditions were described previously [18,19]. Briefly, a parallel plate flow chamber with a peristaltic pump system (Masterflex model 7518-10, Cole-Parmer Instrument Company, Vernon Hills, IL, USA) makes up the shear flow loading system, which can produce a directed steady flow for this study. The loading shear stress is determined using the formula below.

$$\tau = 6\frac{\mu Q}{H^2 W} \quad (1)$$

where τ is the fluid shear stress applied to the cells; μ is the viscosity of the circulating medium; Q is the fluid velocity; H is the chamber height; W is the chamber width.

The shear flow was determined according to the velocity of the circulation medium using the chamber parameters (H = 0.3 mm, W = 95 mm) and the viscosity of the circulating medium (0.83 mPa·s). Liver cancer cells were seeded on a 25 × 75 mm glass slide as a monolayer, and then the slide was placed into the flow chamber when cells with 90% confluent and exposed to a stable 1.4 dyn/cm^2 fluid shear stress (fluid velocity: 14.96 mL/min) for 8 h. The same RPMI-1640 culture medium with 10% fetal bovine serum was supplied to this shear flow loading system and kept at 37 °C in an incubator.

2.3 Condition Medium Collection

To ascertain the effects of soluble factors secreted by liver cancer cells on LX2 activation, we collected different culture mediums of HepG2 cells. Firstly, static condition medium (CM) means the medium extracted from the HepG2 cells after cell starvation overnight without exposure to fluid shear stress, and then the medium was collected and centrifuged at 3000 g (Centrifuge 5804R; Eppendorf, Hamburg, Germany) at 4 °C for 20 min to remove dead cells and cell debris. Secondly, the fluid shear stress-induced medium (FM) means the medium extracted from the HepG2 cells after exposing to the fluid shear stress, and the medium in the loading system was collected and centrifuged at 3000 g, 4 °C for 20 min. The process of collecting condition medium was diagrammed in **Supplementary Fig. 1**.

2.4 Antibodies and Reagents

The detailed information about antibodies used for Western blot analysis and immunofluorescence staining are shown in **Supplementary Table 1**.

2.5 Western Blot Analysis

When the cell growth density reached 80%, the cells were lysed, then the total protein was collected and quantified. Afterward, protein electrophoresis was performed using SDS-PAGE. Following that, transmembrane was conducted to transfer proteins to polyvinylidene difluoride (PVDF) membranes. After that, the membranes were blocked for 3 h in TBST buffer containing 5% BSA at room temperature. The membranes were then treated with primary antibodies overnight at 4 °C and secondary antibodies for 2 h at room temperature. Finally, the bands were visualized by ChemiDocTM XRS+ system, the quantification was performed by Image Lab, and the data were analyzed as well as plotted by GraphPad Prism. All the Western blot analysis were conducted triplicates independently.

2.6 Immunofluorescence Staining and Confocal Microscopy

LX2 cells were treated with CM and FM, respectively for 5 days. And then, the cells were fixed with 4% paraformald ehyde for 10 min, blocked, and permeabilized with 5% BSA (Solarbio, Beijing, China)/0.2% Triton X-100/PBS at room temperature for 60 min. Next, cells were incubated with primary antibodies (FAP, 1:100, diluted in PBS) overnight at 4 °C. After that, fluorescence labeling secondary antibody (1:800, diluted in PBS) was incubated at room temperature for 80 min. Follow that, cells were incubated with phallotoxin (Thermo Fisher Scientific, Waltham, USA.) for 1 h. In the end, the nucleus was stained with DAPI (1:800, diluted in PBS) for 5 min. Olympus confocal microscope (FV1000, Tokyo, Japan) was used to obtain images.

2.7 q-PCR

The method performing qPCR was described previously [19]. The primer sequences were as follows: FAP, 5'-CCAGCAATGATAGCCTCAAG-3' (forward) and 5'-GACCGAAACATTCTGGACTC-3' (reverse); ACTA2, 5'- TCCTTCATCGGGATGGAGTC-3' (forward) and 5'-GGCAATGCCAGGGTACATAG-3' (reverse); PDGFR-β, 5'-TGCGGGACTCGAATTACATC-3' (forward) and 5'-TGGTGTAGAGGCTGTTGAAG-3' (reverse); and GAPDH, 5'-GGATGCAGGGATGATGTTC-3'(forward) and 5'-TGCACCACCAACTGCTTAG-3'(reverse). Gene expression was normalized by GAPDH using the $2^{-\Delta\Delta Ct}$ method.

2.8 MTT Assay

The proliferation ability of LX2 was tested using the MTT assay. Firstly, LX2 cells were grown in CM and FM until 5 days, respectively. The media was then removed, and each well of LX2 cells was treated with 20 μL MTT (5 mg/mL). After that, the 96 well plates were placed in a 37 °C incubator for 4 h. 150 mL/well dimethyl sulfoxide (DMSO) was added to stop the reaction, and the optical density at 490 nm was measured using a microplate reader (Bio-Rad, Hercules, CA, USA).

2.9 Exosome Isolation and Purification

Exosomes were isolated utilizing a combination of centrifugation, ultracentrifugation, and filtration as previous study [25]. Briefly, LX2 culture medium, HepG2 static condition medium, and HepG2 fluid shear stress-induced medium were collected. Then exosomes were isolated by gradient centrifugation as: 300 g $\times$ 20 min, 3000 g $\times$ 20 min, 10,000 g $\times$ 30 min, 100,000 g $\times$ 2 h. All the procedures were at 4 ℃. Finally, 50 μL PBS was used to resuspend the exosomes. The process of exosome isolation is diagrammed **Supplementary Fig. 2**.

2.10 Transmission Electron Microscopy (TEM)

Exosomes suspended in PBS were dropped on the golden grid. Then, exosomes were negatively stained by 2% phosphotungstic acid for 3 min, following air-drying for 15min. Finally, TEM (H-600IV, Japan) at 80 kV was used to observe the exosomes.

2.11 Nanoparticle Tracking Analysis (NTA)

After being diluted in PBS, exosomes were analyzed using the Zeta View (Particle Metrix GmbH, Meerbusch, Germany). To get reliable results, exosome samples were diluted by PBS, ending with approximately 20–100 particles in a field of view.

2.12 Exosome Uptake Assay

The PKH26 (Thermo Fisher Scientific, Waltham, USA) is a kind of red lipophilic dye that could be integrated into the exosome membrane. In this study, freshly isolated exosomes were stained in PKH26 and PKH linker mixture in the dark. Then the labeled exosomes were washed with PBS and purified as the step of 2.9. Finally, labeled exosomes were incubated with cells to verify the exosome uptake.

2.13 Exosome Co-Culture with LX2 Cells

To determine the role of exosomes in activating LX2 cells, exosomes derived from HepG2 static condition medium (CM) and fluid shear stress-induced medium (FM) were isolated and used to treat LX2 cells, respectively. And the exosomes derived from LX2 culture medium were set as control. All the exosomes were added with same concentration (10 μg/mL) to the LX2 cells and co-cultured for 5 days.

2.14 Statistical Analysis

The data was represented as the Mean $\pm$ Standard Error of Mean (SEM) in this study, and GraphPad Prism 6 was used to analyze it. A one-way ANOVA was used to compare multiple groups, followed by Tukey's test. Statistical significance was defined as $p < 0.05$.

3. Results

3.1 Medium from Fluid Shear Stress-Induced Liver Cancer Cells Promoted CAFs Activation

Cancer cells' supernatant contains bioactive components that can influence the activity and phenotypic of recipient cells [1]. Therefore, we hypothesized that the substance released by tumor cells after flow subjection has greater capability of activating fibroblasts through bioactive components. Initially, we collected the conditioned medium from HepG2 cells in the absence or presence of fluid shear stress (FSS) subjection (the medium for HepG2 cells without fluid shear stress was referred to as static condition medium (CM); while the medium for HepG2 cells with fluid shear stress subjection was referred to as fluid shear stress-induced medium (FM)) to study their effect on the activation of hepatic stellate cell LX2. Western blot analyses were conducted to measure the expression of activated fibroblast markers. A slightly increased expression of FAP and α-SMA was observed in LX2 with CM exposure, while the LX2 with FM exposure showed markedly increased expression of FAP and α-SMA (Fig. 1A). Additionally, it exhibited the same trend of expression for FAP, α-SMA, together with PDGFR-β at the gene level (Fig. 1B). Furthermore, the locations and expressions of FAP were investigated by immunofluorescence assay. In line with the data from Western blot analysis, FM markedly increased the FAP expression, while a mild elevates at CM group (Fig. 1C). Moreover, the proliferation of LX2 cells under CM and FM conditions was examined by MTT assays. It was confirmed that FM activated the LX2 cells and elevated their proliferative ability (Fig. 1D). Collectively, LX2 cells cultured with FM, yielded more robust activation than cells cultured with CM and negative control.

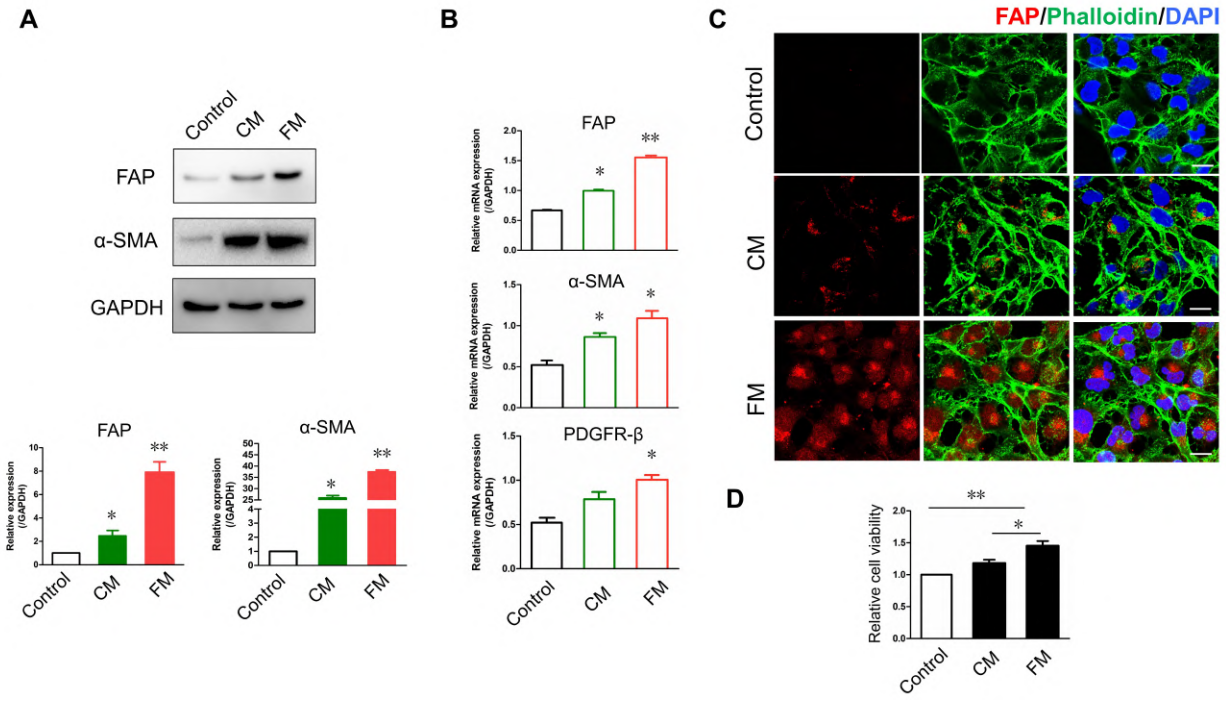

Fig. 1. Fluid shear stress-induced cancer cell medium promoted the activation and proliferation of CAFs. (A) Western-blot analysis of the expression of FAP and α-SMA in LX2 cells after subjecting to static condition medium (CM) or fluid shear stress-induced medium (FM) (n = 3). (B) q-PCR analysis of FAP, α-SMA, and PDGFR-β under the condition of CM or FM (n = 3). (C) Immunofluorescence staining of FAP in LX2 cells treated with CM or FM. Scale bar = 100 μm. Red: FAP; green: phalloidin; blue: DAPI. (D) MTT assay showed the effect of CM or FM on the proliferation of LX2 cells. *$p < 0.05$, **$p < 0$ .01 versus Control group.

3.2 Exosomes Derived from Fluid Shear Stress-Induced Liver Cancer Cells Promote CAFs Activation

The condition medium contained various bioactive substances, such as exosomes, cytokines, and proteins [26,27]. To determine whether exosomes in medium activate CAFs, exosomes derived from HepG2 static condition medium (CM) and fluid shear stress-induced medium (FM) were isolated and used to treat LX2 cells, respectively. And the exosomes derived from LX2 culture medium were set as control. First, the classical methods were used to isolate and identify exosomes in each group [25]. After differential centrifugation and ultracentrifugation, the exosomes from each group were resuspended in PBS, and then validated by transmission electron microscopy (TEM). As shown in Fig. 2A, the exosomes were characterized by the cup-like structure of the membrane, and exosomes with a size range of 50–160 nm were confirmed using nanoparticle tracking analysis (NTA). Furthermore, the vesicles tested positive for exosome markers CD63, Alix and Hsp70 (Fig. 2B).

Next, LX2 cells were incubated with PKH26-labeled exosomes (10 μg/mL) from each group. The PKH26 lipid dye could be observed in each group (Fig. 2C), suggesting that exosomes could be absorbed by LX2 cells. Furthermore, 10 μg/mL exosomes derived from different mediums were added to the LX2 cells and cultured for 5 days. And then we detected the activation of LX2 cells by using Western blot, q-PCR, and immunofluorescence assays after co-culture with CM-exosome (CM-exo) and FM-exosome (FM-exo). All the markers of activated CAFs were upregulated in LX2 cells treated with FM-exo (Fig. 2D–F). The results indicated that LX2 cells cultured with fluid shear stress-induced medium-derived exosomes yielded stronger activation than cells cultured with static condition medium-derived exosomes or negative control-derived exosomes.

3.3 High Level of IGF2 were Detected in Exosomes Derived from Fluid Shear Stress-Induced Liver Cancer Cells

Although IGF2 was upregulated in many cancers as reported previously [23], it generally promotes cancer development in a ligand-receptor way [24,28], and few studies focused on IGF2 delivered by exosomes. We found that the FSS upregulated the expression of IGF2 (Fig. 3A,B) in two liver cells (HepG2 and QGY-7703). Consistently, the significantly increased IGF2 was observed in liver cancer cells exposed to FSS as shown by immunofluorescence (Fig. 3C). Furthermore, the high expression of IGF2 was also detected in the exosome derived from fluid shear stress-induced medium, suggesting that the exosomes from FM contained more IGF2 (Fig. 3D). In addition, recombinant human IGF2 could effectively activate LX2 by upregulating FAP and α-SMA (Fig. 3E).

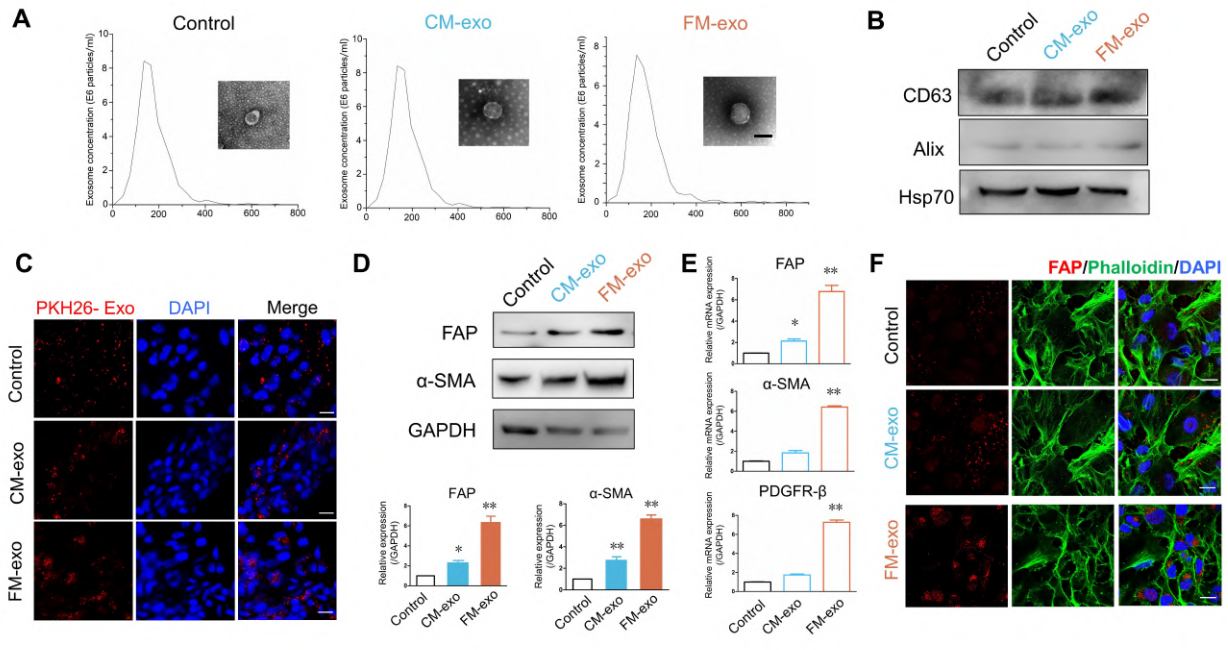

Fig. 2. Exosomes derived from sheared medium promote CAFs activation. Exosomes were isolated from LX2 culture medium (Control), HepG2 static condition medium (CM-exo) and HepG2 fluid shear stress-induced medium (FM-exo), by differential centrifugation and ultracentrifugation, followed NTA and TEM (A) to identify the size and structure of the exosome in each group. Scale bar = 100 μm. (B) Western blot bands of CD63, Alix and Hsp70 in exosomes. (C) Images showing the delivery of PKH26-labeled exosomes (red) to LX2 cells. Scale bar = 100 μm. (D) Western blot bands of FAP and α-SMA level in LX2 cells under the condition of exosomes (10 μg/mL) derived from static condition medium (CM) and fluid shear stress-induced medium (FM) (n = 3). (E) q-PCR analysis of FAP, α-SMA, and PDGFR-β under the condition of exosomes (10 μg/mL) derived from CM and FM (n = 3). (F) Immunofluorescence stating for FAP in LX2 cells when treated with Control, CM-exo and FM-exo (10 μg/mL exosomes). Red: FAP; green: phalloidin; blue: DAPI. Scale bar = 100 μm. *$p < 0.05$, **$p < 0.01$ versus Control group.

3.4 IGF2-PI3K Axis Participated in CAFs Activation

The PI3K/AKT signaling pathway is critical for cell proliferation, differentiation, apoptosis, and mobility [29, 30]. Increasing evidence has identified that PI3K/AKT pathway could promote the differentiation of various cells into CAFs. IGF2 was identified as a key factor that interacted with PI3K pathway to promote carcinogenesis [31]. We previously demonstrated the high level of IGF2 in exosomes derived from fluid shear stress-induced liver cancer cells (Fig. 3D). To investigate the molecular mechanism of flowed-exosome inducing LX2 activation, we examined the IGF2-PI3K-AKT signals in LX2 cells.

As shown in Fig. 4A, exosomes derived from fluid shear stress-induced medium significantly upregulated the expression of IGF2, *p*-PI3K, and *p*-AKT in LX2 cells, compared with the static condition medium group (Fig. 4A). PI3K inhibitor was used to study the role of PI3K in CAFs activation; as expected, the expression of FAP and α-SMA both downregulated, suggesting that PI3K signaling participated in CAFs activation (Fig. 4B). Furthermore, IGF2 is one of the growth factors/cytokines that can activate PI3K/Akt. IGF2 inhibitor, Xentuzumab, was also used to inhibit the activating function of IGF2 in PI3K signal pathway. After inhibiting IGF2, a decreased expression of CAFs activation markers was observed in each group (Fig. 4C).

4. Discussion

Primary liver cancer is a highly fatal disease with high mortality, which has become one of the most malignant tumors and threatens human health [32]. Recent studies strengthened the conception that cross-talk signaling between malignant cells and the liver cancer microenvironment contributed to tumor progression and the pathogenesis of liver cancer [2,33]. As well known, cellular communication in the tumor microenvironment plays a pivotal part in carcinogenic processes; therefore, it necessitates the study of the cellular communication between liver cancer cells and CAFs, especially in the tumor microenvironment (TME).

In recent decades, the physical traits in TME gained more attention. The tumor physical microenvironment, particularly the liquid microenvironment, is particularly crucial in tumor progression because of the pervasive fluid

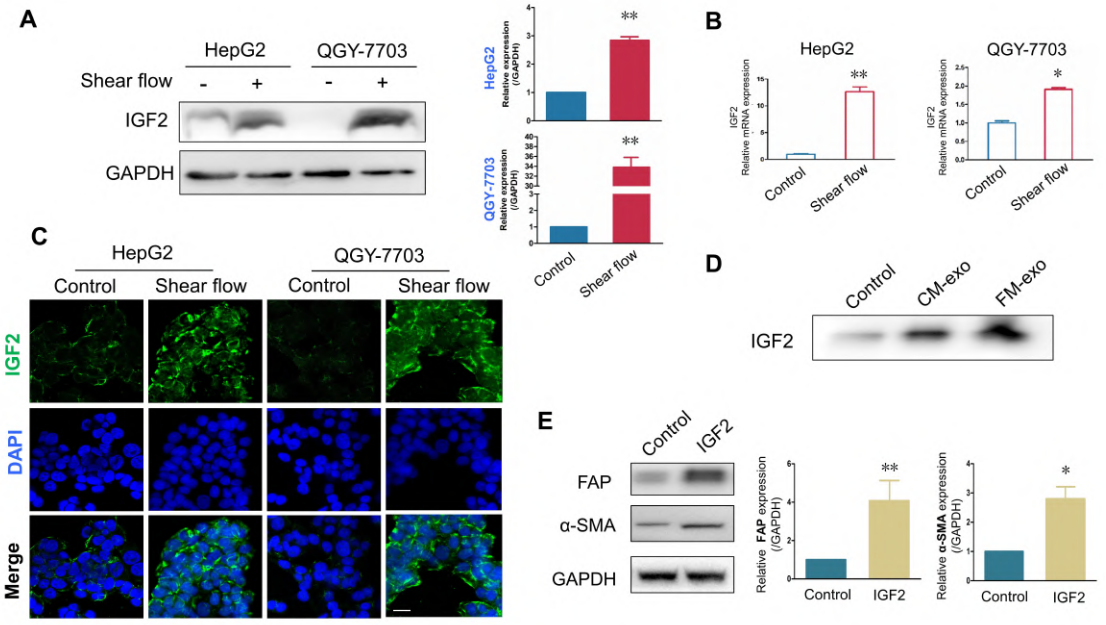

Fig. 3. The expression of IGF2 was upregulated by fluid shear stress in liver cancer cells and exosomes. (A) Images showing the expression of IGF2 in HepG2 and QGY-7703 cells with or without shear flow stimulation, measured by Western blot (n = 3). (B) Graphs showing the expression of IGF2 in HepG2 and QGY-7703 cells, measure by qPCR (n = 3). (C) Images showing the expression of IGF2 in HepG2 and QGY-7703 cells with or without shear flow stimulation, measured by immunofluorescence staining. Scale bar = 100 μm. (D) Image showing the expression of IGF2 in exosomes derived from LX2 medium and CM/FM of HepG2 cells, measured by Western blot. (E) Western blot analysis of the FAP and α-SMA level in LX2 cells with or without recombinant human IGF2 stimulation (n = 3). *$p < 0.05$, **$p < 0.01$ versus Control group.

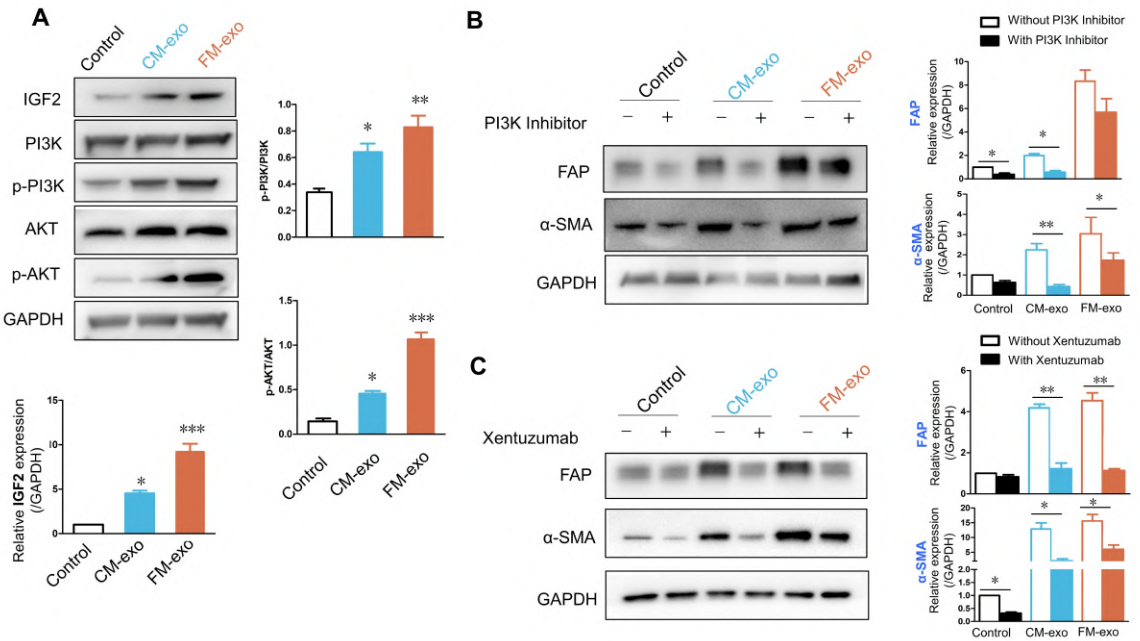

Fig. 4. IGF2-PI3K axis participated in CAFs activation. (A) The expression of key factors in IGF2-PI3K-Akt axis of LX2 treated with exosomes derived from CM or FM (n = 3). (B) The expression of CAFs marker proteins in LX2 cells treated with PI3K inhibitor (n = 3). (C) The expression of CAFs markers in LX2 cells treated with Xentuzumab (n = 3). *$p < 0.05$, **$p < 0.01$ versus Control group.

flow in our bodies [15]. The fluid shear stress affects the biology and function of TME in several ways, including facilitating angiogenesis and lymphangiogenesis [34], regulating matrix metalloproteinase activity as well as cell motility [35], and enhancing cancer cell ability of migration and invasion [36]. Our previous studies have confirmed that elevated fluid shear stress greatly promoted the migration of liver cancer cells [17,18]. In this study, we first analyzed the CAFs activation markers expression both at gene and protein level when typical liver fibroblast cell line LX2 co-culture with condition medium from static HepG2 cell and 1.4 dyn/cm^2 fluid shear stress-induced HepG2 cells. Our data demonstrated that the FM could significantly promote LX2 transform to CAFs, and promote the activation and proliferation ability of LX2 cells (Fig. 1).

Exosomes widely exist in body fluids and are significantly associated with the progression of tumors. Exosomes served as mediators in intercellular communications between various cell types by transporting information cargos, such as nucleic acids, proteins, and lipids, to the recipient cells and changing the recipient cells' behaviors. Therefore, we further investigated if the bioactive substance rests in exosomes and is absorbed by LX2 cells. So, we isolated the exosomes from HepG2 static condition medium and fluid shear stress-induced medium, characterized the exosomes by transmission electron microscopy, nanoparticle tracking analysis and Western blotting, and then they were used to co-culture with LX2. The results showed that the expression of CAFs markers FAP, α-SMA and PDGFR-β in the LX2 increased significantly after being treated with FM-exosome, suggesting that exosome from a flowed condition can induce the activation of LX2.

IGF2, a mitogenic peptide hormone regulating various physiological functions, which is over-expressed in many cancers and is associated with poor prognosis [23]. In the present study, high expression of IGF2 was detected in liver cancer cell lines HepG2 and QGY-7703 cells when treated with 1.4 dyn/cm^2 shear flow. Additionally, exosomes derived from the fluid shear stress-induced HepG2 medium, are also up-expression IGF2. Interestingly, after uptake exosome from FM, LX2 also exhibited elevated IGF2 expression. In addition, blockage of IGF2 downregulates the expression of CAFs activation markers. Plenty of work has shown that PI3K/Akt pathway could promote the differentiation of various cells into CAFs, and upregulated IGF2 could further activate PI3K/Akt signaling [31,37]. Our results further confirmed the participation of PI3K/Akt signaling in CAFs activation. Moreover, the CAFs activation is reduced by PI3K inhibitor.

5. Conclusions

In conclusion, tumor cells in the flowed condition released exosomes are more capable of activating normal fibroblasts to CAFs through transporting IGF2 to the recipient cells and activating the PI3K/Akt signaling. These findings illustrated the fundamental role of intercellular communication in the tumor physical microenvironment and revealed a new molecular mechanism elucidating the activation of CAFs in liver cancer.

Author Contributions

XL and HY conceived the idea and designed the research study. TF and FF performed the research. TL and YS provided help and advice on data analysis. TF, CZ and JH analyzed the data. TF and FF wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

Not applicable.

Funding

This study was supported by grants from the National Natural Science Foundation of China (31971239, 11932014).

Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://www.imrpress.com/journal/FBL/27/3/10.31083/j.fbl2703104.

References

[1] Anderson NM, Simon MC. The tumor microenvironment. Current Biology. 2020; 30: R921–R925.

[2] Santhakumar C, Gane EJ, Liu K, McCaughan GW. Current perspectives on the tumor microenvironment in hepatocellular carcinoma. Hepatology International. 2020; 14: 947–957.

[3] DeBerardinis RJ. Tumor Microenvironment, Metabolism, and Immunotherapy. New England Journal of Medicine. 2020; 382: 869–871.

[4] Alexander J, Cukierman E. Cancer associated fibroblast: Mediators of tumorigenesis. Matrix Biology. 2020; 91–92: 19–34.

[5] Sahai E, Astsaturov I, Cukierman E, DeNardo DG, Egeblad M, Evans RM, *et al*. A framework for advancing our understanding of cancer-associated fibroblasts. Nature Reviews Cancer. 2020; 20: 174–186.

[6] Koyama Y, Brenner DA. Liver inflammation and fibrosis. Journal of Clinical Investigation. 2019; 127: 55–64.

[7] Chen X, Song E. Turning foes to friends: targeting cancer-associated fibroblasts. Nature Reviews Drug Discovery. 2019; 18: 99–115.

[8] Park D, Sahai E, Rullan A. SnapShot: Cancer-Associated Fibroblasts. Cell. 2020; 181: 486–486.e1.

[9] Zhang Z, Karthaus WR, Lee YS, Gao VR, Wu C, Russo JW, *et al*. Tumor Microenvironment-Derived NRG1 Promotes Antiandrogen Resistance in Prostate Cancer. Cancer Cell. 2020; 38: 279–296.e9.

[10] Shiga K, Hara M, Nagasaki T, Sato T, Takahashi H, Takeyama H. Cancer-Associated Fibroblasts: their Characteristics and their Roles in Tumor Growth. Cancers. 2015; 7: 2443–2458.
[11] Wouters BG, Koritzinsky M. Hypoxia signalling through mTOR and the unfolded protein response in cancer. Nature Reviews. Cancer. 2008; 8: 851–864.
[12] Biffi G, Tuveson DA. Diversity and Biology of Cancer-Associated Fibroblasts. Physiological Reviews. 2021; 101: 147–176.
[13] Su S, Chen J, Yao H, Liu J, Yu S, Lao L, *et al*. $CD10^{+}GPR77^{+}$ Cancer-Associated Fibroblasts Promote Cancer Formation and Chemoresistance by Sustaining Cancer Stemness. Cell. 2018; 172: 841–856.e16.
[14] Costa A, Kieffer Y, Scholer-Dahirel A, Pelon F, Bourachot B, Cardon M, *et al*. Fibroblast Heterogeneity and Immunosuppressive Environment in Human Breast Cancer. Cancer Cell. 2018; 33: 463–479.e10.
[15] Nia HT, Munn LL, Jain RK. Physical traits of cancer. Science. 2020; 370: eaaz0868.
[16] Jain RK, Martin JD, Stylianopoulos T. The role of mechanical forces in tumor growth and therapy. Annual Review of Biomedical Engineering. 2014; 16: 321–346.
[17] Yu H, Shen Y, Jin J, Zhang Y, Feng T, Liu X. Fluid shear stress regulates HepG2 cell migration though time-dependent integrin signaling cascade. Cell Adhesion & Migration. 2018; 12: 56–68.
[18] Su G, Feng T, Pei T, Yang F, Sun D, Yu H, *et al*. Autophagy modulates FSS-induced epithelial–mesenchymal transition in hepatocellular carcinoma cells. Molecular Carcinogenesis. 2021; 60: 607–619.
[19] Yu H, He J, Su G, Wang Y, Fang F, Yang W, *et al*. Fluid shear stress activates YAP to promote epithelial–mesenchymal transition in hepatocellular carcinoma. Molecular Oncology. 2021; 15: 3164–3183.
[20] Kalluri R, LeBleu VS. The biology, function, and biomedical applications of exosomes. Science. 2020; 367: eaau6977.
[21] Dai J, Su Y, Zhong S, Cong L, Liu B, Yang J, *et al*. Exosomes: key players in cancer and potential therapeutic strategy. Signal Transduction and Targeted Therapy. 2020; 5: 145.
[22] Wu Q, Zhou L, Lv D, Zhu X, Tang H. Exosome-mediated communication in the tumor microenvironment contributes to hepatocellular carcinoma development and progression. Journal of Hematology & Oncology. 2019; 12: 53.
[23] Livingstone C. IGF2 and cancer. Endocrine-Related Cancer. 2013; 20: R321–R339.
[24] Brouwer-Visser J, Huang GS. IGF2 signaling and regulation in cancer. Cytokine & Growth Factor Reviews. 2015; 26: 371–377.
[25] Zeng Y, Yao X, Liu X, He X, Li L, Liu X, *et al*. Anti-angiogenesis triggers exosomes release from endothelial cells to promote tumor vasculogenesis. Journal of Extracellular Vesicles. 2019; 8: 1629865.
[26] Sandberg TP, Stuart MPME, Oosting J, Tollenaar RAEM, Sier CFM, Mesker WE. Increased expression of cancer-associated fibroblast markers at the invasive front and its association with tumor-stroma ratio in colorectal cancer. BMC Cancer. 2019; 19: 284.
[27] Eckert MA, Coscia F, Chryplewicz A, Chang JW, Hernandez KM, Pan S, *et al*. Proteomics reveals NNMT as a master metabolic regulator of cancer-associated fibroblasts. Nature. 2019; 569: 723–728.
[28] Unger C, Kramer N, Unterleuthner D, Scherzer M, Burian A, Rudisch A, *et al*. Stromal-derived IGF2 promotes colon cancer progression via paracrine and autocrine mechanisms. Oncogene. 2017; 36: 5341–5355.
[29] Yang J, Nie J, Ma X, Wei Y, Peng Y, Wei X. Targeting PI3K in cancer: mechanisms and advances in clinical trials. Molecular Cancer. 2019; 18: 26.
[30] Fruman DA, Chiu H, Hopkins BD, Bagrodia S, Cantley LC, Abraham RT. The PI3K Pathway in Human Disease. Cell. 2017; 170: 605–635.
[31] Cacheux W, Lièvre A, Richon S, Vacher S, El Alam E, Briaux A, *et al*. Interaction between IGF2-PI3K axis and cancer-associated-fibroblasts promotes anal squamous carcinogenesis. International Journal of Cancer. 2019; 145: 1852–1859.
[32] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer Journal for Clinicians. 2018; 68: 394–424.
[33] Wang G, Wang Q, Liang N, Xue H, Yang T, Chen X, *et al*. Oncogenic driver genes and tumor microenvironment determine the type of liver cancer. Cell Death & Disease. 2020; 11: 313.
[34] Song JW, Munn LL. Fluid forces control endothelial sprouting. Proceedings of the National Academy of Sciences of the United States of America. 2011; 108: 15342–15347.
[35] Qazi H, Shi Z, Tarbell JM. Fluid shear stress regulates the invasive potential of glioma cells via modulation of migratory activity and matrix metalloproteinase expression. PLoS ONE. 2011; 6: e20348.
[36] Qazi H, Palomino R, Shi Z, Munn LL, Tarbell JM. Cancer cell glycocalyx mediates mechanotransduction and flow-regulated invasion. Integrative Biology. 2013; 5: 1334–1343.
[37] Badarni M, Prasad M, Golden A, Bhattacharya B, Levin L, Yegodayev KM, *et al*. Igf2 mediates resistance to isoform-selective-inhibitors of the pi3k in hpv positive head and neck cancer. Cancers. 2021; 13: 1–21.