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Article type : Editorial

Cancer-associated fibroblasts: Orchestrating the crosstalk between liver cancer cells and neutrophils via the CLCF1-mediated CXCL6/TGF-β axis

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Abbreviations: CAFs, cancer-associated fibroblasts; CSCs, cancer stem cells; CLCF1, cardiotrophin-like cytokine factor 1; GSEA, gene set enrichment analysis; HCC, hepatocellular carcinoma; PTFs, peritumoral fibroblasts; TANs, tumor-associated neutrophils; TCGA, the Cancer Genome Atlas; TME, tumor microenvironment.

Potential conflict of interest: Nothing to report.

Acknowledgments: This work was supported by the Collaborative Research Fund (C7026-18G), and the Theme-based Research Scheme project (T12-704/16-R).

Liver cancer (hepatocellular carcinoma, HCC) is one of the deadliest diseases and the 4th leading cause of cancer mortality in the world. To prolong the survival of HCC patients, immense efforts have been made to target the intrinsic key signaling pathways regulating the survival of HCC cells. However, the behaviors of cancer cells within the tumor are critically regulated by stromal cells, including fibroblasts, immune cells and endothelial cells, within the tumor microenvironment (TME). Cancer-associated fibroblasts (CAFs) are one of the major cell populations within the TME, and activated fibroblasts are the predominant form of CAFs found in several malignancies. Specifically, in HCC, more than 80% of cases develop within the context of cirrhosis, which is always accompanied by an enrichment of activated fibroblasts due to chronic inflammation.[1] Therefore, it is crucial to understand the potential crosstalk between CAFs and HCC cells.

In 2011, Mazzocca et al. provided the first evidence showing the crosstalk between CAFs and HCC cells. First, they demonstrated the molecular and functional differences between peritumoral fibroblasts (PTFs) and CAFs. Furthermore, they identified tumor-derived lysophosphatidic acid to be crucial in inducing the differentiation of PTFs, which in turn accelerates HCC progression.[2] Evidence that CAF-derived hepatocyte growth factor is capable of regulating the plasticity of liver cancer stem cells (CSCs) through the c-Met/FRA1/HEY1 and AP1/KRT19 signaling pathways and bears clinical significance has also begun to emerge.[3,4] Recently, CAFs were found to recruit immune cells within the TME, including neutrophils and macrophages. For example, CAF-derived endosialin promoted macrophage recruitment and polarization, resulting in HCC progression.[5] In addition, CAFs induced PDL1^{+} tumor-associated neutrophils (TANs) and impaired T cell function via the IL6/STAT3 pathway.[6]

Although crosstalk between CAFs/HCC cells and CAFs/immune cells has been reported, the intricate crosstalk among CAFs, HCC cells, and other cellular components within the TME remains largely unknown, particularly during different HCC stages, which significantly impedes efforts to develop effective cancer therapeutics.

In this issue of HEPATOLOGY, Song et al.[7] reported novel comprehensive cytokine-mediated crosstalk among CAFs, HCC cells and TANs, augmenting cancer stemness and TAN recruitment in HCC. The authors reported for the first time that CAFs isolated from HCC at advanced stages showed a greater tumor-promoting effect *in vivo* than those isolated from early stages. By comparing the expression profiles between PTFs and CAFs, cardiotrophin-like cytokine factor 1 (*CLCF1*) was found to be preferentially expressed and secreted by CAFs, and its levels were correlated with advanced HCC stages. Clinically, high levels of *CLCF1* expression were correlated with aggressive tumor behaviors and poor patient survival. CLCF1 was also previously identified to be preferentially secreted by lung CAFs with clinical significance.[8] Upon gene set enrichment analysis (GSEA), *CLCF1* expression showed a negative correlation with stem cell differentiation in a cohort of HCC samples. Along with these data, the authors found that CAF-derived CLCF1 enhanced cancer stemness in HCC, and the effect was mitigated by repressing the expression of CNTFR in HCC cells. Additionally, this study uncovered a mechanism underlying the CSC-enhancing role of CAF-derived CLCF1 in HCC. As a novel inflammatory cytokine, CLCF1 was found to upregulate two key cytokines, CXCL6 and TGF-β, via the Akt/ERK1/2/STAT3 signaling axis. Mechanistically, CXCL6 was found to regulate cancer stemness via the transcriptional regulation of stemness-associated genes, including *SOX2*, via E2F1, while TGF-β affected its CSC-enhancing effect via the p38 pathway. Interestingly, the suppression of STAT3 fully abolished the CSC-enhancing effect of CLCF1, while the addition of CXCL6 and TGF-β only partially restored this suppressive effect, suggesting that STAT3 is the major effector of CLCF1-mediated cancer stemness in HCC. This result is in line with a previous report showing the critical involvement of STAT3 in the regulation of liver CSCs in HCC.[9]

As TANs are one of the most infiltrated immune cells within the TME of HCC, it is possible that there is potential crosstalk between CAFs and TANs. Another interesting result is that Song et al. dissected the interactions among CAFs, HCC and TANs, which are mediated via a cytokine network. Data from the Cancer Genome Atlas (TCGA) database showed that CXCL6 was significantly correlated with CD15, the cell surface marker of neutrophils, while TGF-β displayed a strong association with MMP9, a functional marker of N2-polarized neutrophils. Based on this interesting finding, the authors performed a functional study and found that recombinant CXCL6 and TGF-β function to recruit and induce the "N2" polarization of TANs, respectively. This result, together with the data showing the enhanced recruitment of TANs upon the treatment of HCC cells with either CLCF1 or CAF supernatant, suggests the role of CAF-derived CLCF1 in recruiting and inducing the "N2" polarization of TANs via the induced secretion of CXCL6 and TGF-β in HCC cells. However, since TGF-β also induced the activation, maturation and differentiation of other immune cells, including NK cells, macrophages, dendritic cells, and T cells, there is also a possibility that other immune cells may also be involved in the CLCL1-mediated signaling cascade.

Additionally, this study provided mechanistic insight into how HCC cells educate CAFs to enhance their potential to regulate cancer stemness and the recruitment of TANs. Song et al. showed that ERK1/2 phosphorylation was associated with CLCF1 expression, and their levels in CAFs were higher when isolated from late-stage HCC tissues than from early-stage HCC tissues. Furthermore, they found that ERK1/2 signaling in CAFs is required for CLCF1 expression in CAFs, which is activated by HCC-secreted CXCL6 and TGF-β in a paracrine manner. Using an *in vivo* mouse model, they were able to confirm the functional role of CLCF1 in coordinating the crosstalk among CAFs, HCC cells, and TANs through an ERK1/2-induced CLCF1/CNTFR positive feedback loop. In a cohort of HCC clinical samples, they showed the role of the CLCF1-CXCL6/TGF-β axis in the regulation of cancer stemness, and the recruitment of "N2" TANs was clinically relevant, which contributes to the poor prognosis of HCC patients.

In summary, this study provides a novel CLCF1-driven CXCL6/TGF-β/ERK1/2 positive feedback loop involving crosstalk among different cellular components, including CAFs, HCC cells and TANs (Fig. 1). Accordingly, this study opens a new therapeutic avenue for targeting HCC by the selective blockade of CLCF1/CNTFR or ERK1/2 signaling, warranting a thorough investigation.

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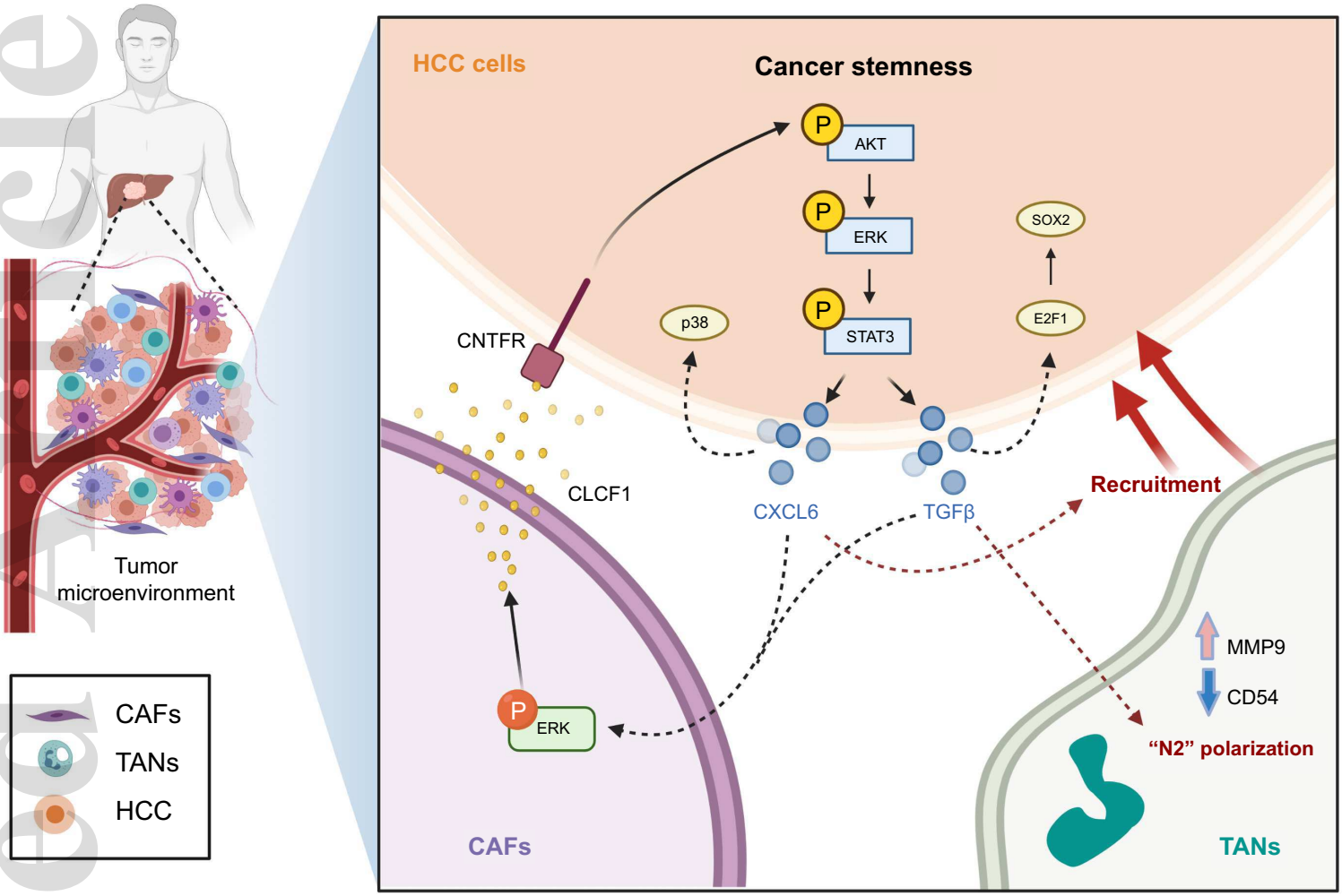
Figure Legend

Fig. 1. Schematic representation of the cytokine-mediated crosstalk among CAFs, HCC cells and TANs.



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