



**HAL**  
open science

## Cancer-associated fibroblasts in cholangiocarcinoma

Javier Vaquero, Lynda Aoudjehane, Laura Fouassier

► **To cite this version:**

Javier Vaquero, Lynda Aoudjehane, Laura Fouassier. Cancer-associated fibroblasts in cholangiocarcinoma. *Current Opinion in Gastroenterology*, 2020, 36 (2), pp.63-69. 10.1097/MOG.0000000000000609 . hal-02746870

**HAL Id: hal-02746870**

**<https://hal.science/hal-02746870>**

Submitted on 16 Nov 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

## **Cancer-associated fibroblasts in cholangiocarcinoma**

Javier Vaquero <sup>1,2</sup>, Lynda Aoudjehane<sup>1,3</sup>, and Laura Fouassier <sup>1</sup>

1 Sorbonne Université, Inserm, Centre de Recherche Saint-Antoine, CRSA, Paris, France

2 LPP, CNRS, Ecole Polytech., Univ. Paris-Sud, Observatoire de Paris, Univ. Paris-Saclay, Sorbonne Université, PSL Research University, Paris, France.

3 Institute of Cardiometabolism and Nutrition, ICAN Paris, France

Author of correspondence:

Laura Fouassier, Ph.D.

INSERM Sorbonne Université, UMRS\_938

Centre de Recherche Saint-Antoine

27 rue Chaligny

75012 Paris France

33 6 98 77 40 01

[laura.fouassier@inserm.fr](mailto:laura.fouassier@inserm.fr)

**Abstract (200w)**

Cholangiocarcinoma is a biliary tumor with a high contingency of cancer-associated fibroblasts (CAF) Purpose of review:

Recent findings:

Summary:

**Keywords:**

3-5 keywords

## Text of review (2500 words maximum)

### Introduction:

Tumor is a complex tissue with intricate acellular and cellular components. Even if tumor cells have the main role, tumor growth and progression cannot be accomplished alone without extracellular matrix (ECM) and stromal cells. The non-tumor cell contingent includes fibroblasts, named cancer-associated fibroblasts (CAF), immune and endothelial cells. Altogether, the non-tumor cells and ECM creates a fertilizing microenvironment for tumor cells. Cholangiocarcinoma, a biliary tract cancer with aggressive properties is the prototype of tumor containing an abundant desmoplastic stroma, with high amount of CAF and ECM. CAF in CCA, irrespective of the subtype, have not been fully characterized with the exception of some signaling pathways that play a role in tumor progression thanks to their reciprocal communication with tumor cells. Based on more in-depth studies of other cancers, we aim to overview new vision of the CAF functions in CCA.

### I. Origin and phenotypic heterogeneity of CAF

Origin of CAF in CCA remains unknown in CCA but according to liver structure, the major cell sources of CAF are believed naturally to be hepatic stellate cells (HSC) and portal fibroblasts (PF), resident fibroblasts localized in portal tract {Lemoine, 2015 #8394} revue à mettre 27324023. Even if the HSC are known to be the major source of myofibroblasts in biliary diseases, portal fibroblasts owing their localization in porta tract close to bile ducts, may represent also a non-negligible source of CAF in CCA. Indeed, expression of PF markers (i.e. fibulin-2) have been found in CCA CAF, in addition to HSC markers such as desmin and GFAP (glial fibrillary acidic protein) (voir avec Chantal) in  $\alpha$ -SMA-positive CAF, strongly suggesting that both HSC and PF may give rise to CAF in CCA {Itou, 2019 #8389} {Okabe, 2009 #6832}. Other sources of CAF in CCA may exist either outside or inside the tumor such as bone marrow-derived mesenchymal precursor cells or tumor cells, i.e. tumor cells undergoing epithelial-mesenchymal transition but this last option has been dismissed in CCA {Cadamuro, 2013 #7673} and pancreatic cancer, another desmoplastic tumor {Ohlund, 2017 #8396}. Whether CAF in CCA originate from endothelial cells through endothelial-to-mesenchymal transition remains unknown. At this stage, cell fate tracing is required to define the origin of CAF in CCA.

Neither the myofibroblastic cell strain nor the cholangiocarcinoma cell strain were immunoreactive for either CD45 or for CD34, suggesting that hematopoietic stem cells or bone marrow-derived fibrocytes do not function as progenitors of the TDF<sub>SM</sub> or TDE<sub>CC</sub> cell types (Article de sirica AJP).

Besides the origin of CAF defined as activated fibroblasts or myofibroblasts, how the HCS/resident fibroblasts or circulating mesenchymal cells are activated (attracted and stimulated)? One hypothesis is the education of fibrogenic cells by cancer cells, leading for example stellate cells to adopt a CAF phenotype. This concept has recently been supported by several studies performed in pancreatic cancer, in which stellate cells are educated by tumor cells, providing CAF subtype populations {Neuzillet, 2019 #8400} {Ohlund, 2017 #8396} {Xu, 2010 #8411}. More interestingly, once activated, CAF phenotype can be reversed reflecting the high plasticity of these cells. To date, the notion of CAF subtypes is obvious and could explain the multiple functions attributed to CAF, among them ECM remodeling, tumor cell growth and invasion, angiogenesis and immune modulation. The multiple CAF populations may thereby contribute to the stroma heterogeneity and explain their ambivalent functions, pro or anti-tumor. Heterogeneity of CAF has been well illustrated especially in pancreas {Ohlund, 2017 #8396} {Neuzillet, 2019 #8400} and breast {Costa, 2018 #8395} cancers, and remains unexplored in CCA.

Identification of CAF subtypes using specific markers is challenging and currently, it is based on well-known fibroblast markers. The most known marker used to identify CAF is  $\alpha$ -SMA (alpha-smooth muscle actin), a marker of smooth muscle cells features that characterize CAF define as activated fibroblast or myofibroblast. However, it has been shown that some CAF populations do not express  $\alpha$ -SMA or at low level {Ohlund, 2017 #8396} {Neuzillet, 2019 #8400} {Costa, 2018 #8395}. Other known fibroblast markers including FAP (fibroblast activation protein), integrin- $\beta$ 1/CD29, S100A4/FSP1 (fibroblast specific protein-1), PDGFR- $\beta$  (platelet derived growth factor receptor-beta) and CAV-1, are regularly used for immunostaining or isolate CAF. Using this marker panel, Costa and al. have been able to identify four populations of CAF in human breast cancer {Costa, 2018 #8395}. In pancreas cancer, Neuzillet et al. have been also able to identify four main population of CAF by unsupervised approach, indicating there are more than one population of CAF in tumor. In CCA,  $\alpha$ -SMA, is the referential marker to evaluate the presence of CAF, at least the CAF population that express this marker. Indeed, distinct subpopulation of CAF either with high or low expression of  $\alpha$ -SMA have been identified in pancreatic cancer. Interestingly these two populations display distinct localization and function;  $\alpha$ -SMA-positive CAF population, also positive for FAP, another marker of myofibroblasts are localized nearby cancer cells, and an  $\alpha$ -SMA-negative/low CAF population

localized further away from tumor cells with a cytokine secretory profile (producing IL6) {Ohlund, 2017 #8396}.

Other typical fibroblast markers have been also found in CAF of CCA including S100A4/FSP1, Thy-1/CD90 and PDGFR-b, with a higher expression in CAF than in non-tumor fibroblasts (from adjacent tumor) {Itou, 2019 #8389}{Utispan, 2010 #7151}{Zhang, 2017 #8421}. However, contradictory results have been obtained recently, in which an expression of CD90 in CAF of iCCA has not been found {Yamaoka, 2018 #8420}. Functionally, CCA CAF, but not non-tumor fibroblasts, promotes tumor growth both *In vitro* and *in vivo*. As expected, expression of  $\alpha$ -SMA in CAF is a predictor of poor prognosis and correlates with clinicopathological factors including tumor size, grade and the presence of lymph node metastasis, emphasizing their role in tumor progression {Sha, 2018 #8413}{Chuaysri, 2009 #6827}. The proportion of  $\alpha$ -SMA-positive CAF in iCCA has been estimated by morphometric analysis, and the average is  $\approx$ 11-13 % {Massani, 2013 #7833}{Cadamuro, 2013 #7673}. Among other markers, CD10 (neprilysine) is a membrane-bound metalloprotease expressed by CAF in CCA, but its expression is more frequently observed in pCCA and eCCA compared to iCCA. In addition, CD10 expression in CCA is correlated with lower tumor differentiation {Nishihara, 2009 #7909}. Podoplanin, a membrane mucin-type protein, has been identified in CAF of pCCA and iCCA, and its expression is associated with lymph node metastasis, lymphatic vessel density and poor outcome {Obulkasim, 2018 #8417}{Hu, 2018 #8422}{Cadamuro, 2019 #8399}. Periostin is a secreted extracellular matrix protein mainly expressed by CAF in CCA {Utispan, 2010 #7151}.

CAF are activated fibroblasts called myofibroblasts (MF) that synthesize ECM components like fibroblasts, in surrounding stroma and display features of smooth muscle cells by expressing  $\alpha$ -SMA coded par *ACTA* gene, conferring higher contractility to MF than fibroblasts. A well-known activator of  $\alpha$ -SMA promoter is smad3, which is part of TGFB-dependent signaling pathway. In CCA, a genomic signature of TGFB has been identified in stromal compartment {Sulpice, 2013 #7288}{Andersen, 2012 #7311}, indicating that TGFB signaling has a major role in  $\alpha$ -SMA induction and therefore in fibroblast activation as demonstrated in 3D organotypic culture model of iCCA established from a rat model of CCA (Sirica AE).

## II. Pleiotropic functions of CAF

CCA stroma is a reservoir of factors as demonstrated by the transcriptomic analysis {Sulpice, 2013 #7288}{Andersen, 2012 #7311}. At the cell level, CAF is a cell factory that may account for the production of most stroma components; they produce growth factors, cytokines, metabolites and ECM proteins regulating tumor cell functions, immunity and angiogenesis. Table 1 reports molecules synthesized by CCA CAF, evidenced either by immunostaining on tissue or at mRNA level in isolated cells. Among these factors, ligands of EGFR and PDGFR, chemokines (SDF-1/CXC12, ), angiotensin II, proteases and ECM proteins (periostin) are expressed by native CAF. Besides factor, CAF produce extracellular vesicles that are now recognized as a major cell-cell communication mediator {Maia, 2018 #8425}. In a cell model of CAF, LX2 (myofibroblast derived from human hepatic stellate cells), a release of EVs containing microRNA-195 has been shown {Li, 2017 #8424}. By producing diverse molecules and EV, CAF are able to communicate not only with tumor cells but also with stromal cell conferring a central role in tumor progression and therapeutic response. Most studies of the studies presented here deals with human myofibroblasts derived from hepatic stellate cells, either primary cells or cell lines (such LX-2 and LI-90).

### **CAF regulate proliferation, survival and migration of tumor cells in CCA**

SDF-1 (also named CXCL12) is chemokine that was early identified in native CCA CAF {Ohira, 2006 #6834}. In this first study, SDF1-1 is mainly expressed in native (or primitive) CAF in human iCCA, and to a less extent in tumor cells, while its receptor, CXCR4, is mainly expressed by tumor cells {Ohira, 2006 #6834}{Gentilini, 2012 #7005}. By using cell 2-D cell culture model with cell lines (fibroblasts and cancer cells) recapitulating the interplay between CAF and tumor cells, it was demonstrated that SDF-1 is expressed by myofibroblasts and produce in cell supernatant to stimulate CCA cell migration through activation of ERK and AKT pathways {Ohira, 2006 #6834}{Gentilini, 2012 #7005}. However, SDF-1 doesn't have any effect on CCA cell proliferation {Ohira, 2006 #6834}{Gentilini, 2012 #7005} but instead acts as a pro-survival factor when cells are challenged with an apoptotic inducer {Gentilini, 2012 #7005}. Besides its action on tumor cell, SDF-1 stimulate activation and proliferation of hepatic HSC in an autocrine way {Okamoto, 2012 #7106}. MCP-1 (monocyte chemoattractant protein-1/CCL2) is another chemokine produced by myofibroblast derived from HSC and CCA-CAF that contributes to CCA cell motility {Gentilini, 2012 #7005}{Heits, 2016 #8433}.

PDGF/PDGFR axis is far away the most studied pathway that contributes to the interplay between tumor cells and CAF in CCA {Brivio, 2017 #8128}. Several ligands of PDGFR have been evidenced in native CAF of CCA (see table 1) and hepatic myfibroblast cell lines. PDGF-BB was the first identified by immunohistochemistry in CAF of human CCA {Fingas, 2011 #6861}. Since, PDGF-A and -D were also identified in CAF {Rizvi, 2014 #8426}. As demonstrated for SDF-1, PDGF-BB derived from myfibroblasts promotes resistance to death in CCA cells, a cytoprotection that relies on hedgehog pathway {Fingas, 2011 #6861}. Although tumor cells express PDGFR $\beta$  and respond to its ligands through a paracrine way, CAF also express this receptor {Cadamuro, 2013 #7673}. Thus, it has been shown that PDGF-D secreted by CCA cells stimulate myfibroblast migration and to a less extent proliferation, suggesting that PDGF-D is responsible for the recruitment of myfibroblasts into the tumor {Cadamuro, 2013 #7673} but also induces VEGF-A and -C secretion in myfibroblasts, leading lymphoangiogenesis and lymphatic invasion. In addition, it has been shown that PDGF-D, but also PDGF-b, increases the sensitivity of hepatic myfibroblasts and not of tumor cells to navitoclax-mediated apoptosis {Rizvi, 2014 #8426}, indicating that PDGF is involved, by increasing the level of the proapoptotic protein Puma, in CAF priming to apoptosis. With higher susceptibility to apoptosis, CAF could be specifically targeted opening new area for CCA treatment {Mertens, 2013 #7178}. Indeed, BH3 mimetics, ABT-199 or navitoclax, reduce tumor growth in murine CCA model by depleting myfibroblasts from the tumor microenvironment {Mertens, 2013 #7178}{Rizvi, 2014 #8426}.

EGFR pathway is essential for CCA biology {Pellat, 2018 #8427}. Dysregulations of EGFR expression and signaling have been associated with development and progression of CCA. While the pathway has been widely studied in cancer cells, its role in the interplay between cancer cells and CAF has been less investigated. CAF from CCA express EGFR ligands including HB-EGF, EGF, amphiregulin and epiregulin (see Table 1). In a previous study, we demonstrated that the cotransplantation of CCA cells with human liver myfibroblasts into immunocompromised mice increased tumor incidence, size, and metastatic dissemination of tumor through EGFR pathway. *In vitro*, production of EGFR ligands by myfibroblasts activate EGFR expressed at the tumor cell surface, promoting migration and invasion. Interestingly, EGFR stimulation promotes expression of TGF $\beta$ , which in turn triggers the expression of EGFR ligands by stromal cells {Claperon, 2013



#7672}. The contribution of EGFR signaling in the interplay between CAF and tumor cells has been also emphasized in CAF, from a preclinical rat model of CCA, that express EGFR ligands TGF- $\alpha$  and amphiregulin {Manzanares, 2017 #8140}.

IGF2/IGF1R/IR axis was recently outlined by our team as a signaling pathway involved in resistance to anti-EGFR therapy in CCA. More importantly, we discovered that IGF2 is expressed by CCA CAF and acts through paracrine and autocrine ways. First, by acting on tumor cells, IGF2 activates IGF1R and IR and induces cell plasticity driving resistance to anti-EGFR. Second, by acting of CAF, IGF2 promotes cell proliferation, contributing presumably to the desmoplastic reaction and to the amplification of IGF2/IGF1R/IR pathway in tumor cell maintaining thereby the pressure of drug resistance {Vaquero, 2018 #8309}.

Periostin is an extracellular matrix protein produced by  $\alpha$ -SMA+ CAF in CCA {Utispan, 2010 #7151} {Darby, 2010 #8416} {Sirica, 2014 #7823}. Expression of periostin is higher in iCCA compared to non-tumor counterpart {Mino, 2017 #8430}, and is associated with poor prognosis and malignant progression in human and rat CCA model {Utispan, 2010 #7151} {Manzanares, 2018 #8429}. Receptors of periostin comprise integrins expressed at the tumor cell surface. By binding integrin  $\alpha 5\beta 1$  on CCA cells, periostin stimulates invasion through an epithelial-mesenchymal transition (EMT) process {Utispan, 2012 #8432} {Mino, 2017 #8430}.

### **CAF regulate angiogenesis and immunity**

Besides the interplay between CAF and tumor cells, CAF have the ability to produce factors that influence neighboring stromal cells. Comparative protein expression performed by a protein-array approach has identified VEGF-A as elevated in iCCA compared to pCCA {Guedj, 2009 #3648}. As a potential source of VEGF-A, CAF from iCCA have been identified. Indeed, upon PDGF-D stimulation, CAF produce VEGF-A and VEGF-C resulting in the expansion of lymphatic vasculature and tumor cell intravasation {Cadamuro, 2019 #8399}. Little is known regarding whether or not CAF are producing angiogenic factors other than VEGF. A recent proteomic study has evidenced multiple proangiogenic factors such as neuropilins (NRP2), VCAM1, PECAM1, VWF, and PDGFR beta in human iCCA emerging from a cirrhotic liver compared to normal liver {Le Faouder, 2019 #8397} without knowing the cellular origin of these factors.

CAF communicate with tumor infiltrating immune cells to drive an immunosuppressive microenvironment {Monteran, 2019 #8436} and many studies toward this function are now well established with the identification of sub-population of CAF driving immunosuppression functions {Costa, 2018 #8395} {Ohlund, 2017 #8396}. CAF from CCA produce factor that modulate innate immune response such as IL-8, IL-13, MCP-1/CCL2, MIF (Macrophage migration inhibitory factor) {Heits, 2016 #8433} {Hogdall, 2018 #8435}. IL-13 has been recently identified in CCA, among other factors, responsible for macrophage-differentiation and invasion, as well as for *in vivo* tumor-promoting effect {Raggi, 2017 #8434}. In CCA, a FAP-STAT3-CCL2 signaling has been identified in CAF, sufficient to induce an inflammatory program through the recruitment of myeloid-derived suppressor cells (MDSC) which are characterized by immunosuppressive activity {Yang, 2016 #8398}. The expression of immune checkpoint molecules has been evidenced in CAF from pancreatic tumors, compromising the proliferative and functionality of T-cells {Gorchs, 2019 #8437}. Although immune check points are expressed by tumor and inflammatory cells {Fontugne, 2017 #8351}, there is no report so far regarding their expression in CCA-CAF.

### III. CAF and cancer treatment

Treatment efficacy in CCA is a constant challenge for clinicians, and the vision to target CAF instead tumor cells is a new opening field for cancer treatment. In CCA, several strategies can be proposed:

#### Depletion

Targeted therapieIn CCA, the pioneer work in this field comes for Gores' work. One strategy developed by his group was to deplete CAF from the tumor by using Navitoclax, a potent and oral Bcl-2 family protein inhibitor that binds to multiple anti-apoptotic Bcl-2 family proteins. Navitoclax induces apoptosis of CAF but has no effect on quiescent fibroblast or CCA cells {Mertens, 2013 #7178}.

#### TGFB

Partie TGFB a metre dans origin .. Manzanares AJP 2017 TGFB. That treatment of the 3D TDE<sub>CC</sub> þ TDF<sub>SM</sub> cell co-cultures with the selective TGF-b receptor 1 serine/ threonine kinase inhibitor LY2157299 led to a prominent dose-dependent attenuation of the dense fibrocollagenous matrix accompanied by significant decreases in the numbers of a-SMAepositive TDF<sub>SM</sub> myofibroblastic-like cells within the gel matrix most clearly suggests a model in which the TGF-b pathway functions as a major contributor to the development of desmoplastic stroma in mass-forming ICC. Some limited studies have suggested that targeting the TGF-b pathway and hepatic stellate cell activation may prevent cholangiocarcinoma development. For example, halofuginone, when administered orally to rats before thioacetamide-induced liver fibrosis, has been reported to prevent hepatic stellate cell activation, and more relevant to our current results, when given to rats with established fibrosis,

halofuginone was found to cause complete resolution of the fibrotic condition.<sup>66</sup> Halofuginone was also determined to prevent pancreatic xenograft tumor development in nude mice by inhibiting pancreatic stellate cell activation.<sup>67</sup> In addition, murine neutralizing TGF- $\beta$  monoclonal antibody 1D11, when administered to rats with established hepatic fibrosis induced by thioacetamide, was shown to result in a regression of tissue injury and fibrosis when compared with livers from vehicle control-treated animals. This antifibrotic effect was further found to be concomitantly associated with a reduction in the development of cholangiocarcinomas formed in the thioacetamide-treated rats.<sup>68</sup>

[Influence of mTOR-inhibitors and mycophenolic acid on human cholangiocellular carcinoma and cancer associated fibroblasts.](#) Heits N, Heinze T, Bernsmeier A, Kerber J, Hauser C, Becker T, Kalthoff H, Egberts JH, Braun F. *BMC Cancer*. 2016 May 20;16:322. doi: 10.1186/s12885-016-2360-8.

### **Resistance mechanisms (IGF2)**

Select item 2427783411.

[Targeting CXCL12 from FAP-expressing carcinoma-associated fibroblasts synergizes with anti-PD-L1 immunotherapy in pancreatic cancer.](#) Feig C, Jones JO, Kraman M, Wells RJ, Deonarine A, Chan DS, Connell CM, Roberts EW, Zhao Q, Caballero OL, Teichmann SA, Janowitz T, Jodrell DI, Tuveson DA, Fearon DT. *Proc Natl Acad Sci U S A*. 2013 Dec 10;110(50):20212-7. doi: 10.1073/pnas.1320318110. Epub 2013 Nov 25. PMID:24277834

Holofuginone anti-TGF

### **The Tumor Microenvironment: A Druggable Target for Metastatic Disease? Commentaire de l'article publié dans cancer cell par fatima mechta gregoriou**

[EBioMedicine](#).  
PMID:29759482

Immune cells are recruited to the tumor by neoantigens generated by the mutational load of cancer cells. The presence of tumor-infiltrating lymphocytes (TILs) is associated with tumor regression and better patient prognosis. However, the TME provides an immunosuppressive environment via activation of inhibitory T cell receptors on cancer cells which can dampen the TIL response. Immunosuppression by the tumor is not only regulated by cancer cells but also, as recently demonstrated by Mechta-Grigoriou, and colleagues in *Cancer Cell*, by specific subgroups of CAFs. In this study, different subgroups of CAFs were identified in breast cancer which exhibited immunosuppressive features by attracting and retaining TILs to the stroma rather than releasing them into the tumor mass. This exemplifies the complexity and dynamic nature of the TME and how the entire cellular ecology can affect the responses of one cell type.

- [Rechercher](#)

[\[PDF\] sciencemag.orgFree from Publisher](#)

[Tensile forces drive a reversible fibroblast-to-myofibroblast transition during tissue growth in engineered clefts](#)

[P Kollmannsberger, CM Bidan... - Science ..., 2018 - advances.sciencemag.org](#)

Myofibroblasts orchestrate wound healing processes, and if they remain activated, they drive disease progression such as fibrosis and cancer. Besides growth factor signaling, the local extracellular matrix (ECM) and its mechanical properties are central regulators of these processes. It remains unknown whether transforming growth factor- $\beta$  (TGF- $\beta$ ) and tensile forces work synergistically in up-regulating the transition of fibroblasts into myofibroblasts and whether myofibroblasts undergo apoptosis or become deactivated by other means once ...

## Metabolic reprogramming

MEK inhibitors impact CCA stroma (Wang P)

### Conclusion

Emerging functions of CAF : modulator tumor metabolism (metabolic symbiosis between CAF and tumor cells)

Exchange of cellular material with tumor cells through EV (Li L Hepatolog 2017, CCA rat model)  
Epigenetic

### [Fibroblasts as Modulators of Local and Systemic Cancer Metabolism.](#)

Sanford-Crane H, Abrego J, Sherman MH.

Cancers (Basel). 2019 May 3;11(5). pii: E619. doi: 10.3390/cancers11050619. Review.  
PMID:31058816

Uttipisan 2010 Mol cancer :

a reverse Warburg effect in tumor tissues, in which host cells such as CAFs have been found to "fuel" the cancer cells via metabolites transfer. Tumor-associated stromal components can support tumor growth by providing nutrients that supplement those provided by the local vasculature.

Guido 2012 22874531 Metabolic reprogramming of cancer-associated fibroblasts by TGF- $\beta$  drives tumor growth: connecting TGF- $\beta$  signaling with "Warburg-like" cancer metabolism and L-lactate production.

[Transforming growth factor- \$\beta\$ : guardian of catabolic metabolism in carcinoma-associated fibroblasts.](#) [Cell Cycle. 2012]

### [Tumor-Stroma Mechanics Coordinate Amino Acid Availability to Sustain Tumor Growth and Malignancy.](#)

Bertero T, Oldham WM, Grasset EM, Bourget I, Boulter E, Pisano S, Hofman P, Bellvert F, Meneguzzi G, Bulavin DV, Estrach S, Feral CC, Chan SY, Bozec A, Gaggioli C. Cell Metab. 2019 Jan 8;29(1):124-140.e10. doi: 10.1016/j.cmet.2018.09.012. Epub 2018 Oct 4. PMID:30293773

### [Cancer-associated fibroblasts promote prostate cancer malignancy via metabolic rewiring and mitochondrial transfer.](#)

Ippolito L, Morandi A, Taddei ML, Parri M, Comito G, Iscaro A, Raspollini MR, Magherini F, Rapizzi E, Masquelier J, Muccioli GG, Sonveaux P, Chiarugi P, Giannoni E. Oncogene. 2019 Jul;38(27):5339-5355. doi: 10.1038/s41388-019-0805-7. Epub 2019 Apr 1. PMID:30936458

### [Oxidized ATM-mediated glycolysis enhancement in breast cancer-associated fibroblasts contributes to tumor invasion through lactate as metabolic coupling.](#)

Sun K, Tang S, Hou Y, Xi L, Chen Y, Yin J, Peng M, Zhao M, Cui X, Liu M. EBioMedicine. 2019 Mar;41:370-383. doi: 10.1016/j.ebiom.2019.02.025. Epub 2019 Feb 22. PMID:30799198

[Cancer-associated fibroblasts enhance tumor <sup>18</sup>F-FDG uptake and contribute to the intratumor heterogeneity of PET-CT.](#) Shangguan C, Gan G, Zhang J, Wu J, Miao Y, Zhang M, Li B, Mi J. *Theranostics*. 2018 Feb 2;8(5):1376-1388. doi: 10.7150/thno.22717. eCollection 2018. PMID:29507627

[Cancer-associated fibroblasts promote prostate cancer malignancy via metabolic rewiring and mitochondrial transfer.](#) Ippolito L, Morandi A, Taddei ML, Parri M, Comito G, Iscaro A, Raspollini MR, Magherini F, Rapizzi E, Masquelier J, Muccioli GG, Sonveaux P, **Chiarugi P**, Giannoni E. *Oncogene*. 2019 Jul;38(27):5339-5355. doi: 10.1038/s41388-019-0805-7. Epub 2019 Apr 1. PMID:30936458

[Cell Cycle](#). 2012 Dec 1;11(23):4302-3. doi: 10.4161/cc.22811. Epub 2012 Nov 16.

**Transforming growth factor- $\beta$ : guardian of catabolic metabolism in carcinoma-associated fibroblasts.**

- [Format: Abstract](#)

[Send to](#)

[Cell Metab](#). 2019 Jan 8;29(1):124-140.e10. doi: 10.1016/j.cmet.2018.09.012. Epub 2018 Oct 4. Tumor-Stroma Mechanics Coordinate Amino Acid Availability to Sustain Tumor Growth and Malignancy. [Bertero T](#)<sup>1</sup>, [Oldham WM](#)<sup>2</sup>, [Grasset EM](#)<sup>3</sup>, [Bourget I](#)<sup>3</sup>, [Boulter E](#)<sup>3</sup>, [Pisano S](#)<sup>3</sup>, [Hofman P](#)<sup>4</sup>, [Bellvert F](#)<sup>5</sup>, [Meneguzzi G](#)<sup>3</sup>, [Bulavin DV](#)<sup>6</sup>, [Estrach S](#)<sup>3</sup>, [Feral CC](#)<sup>3</sup>, [Chan SY](#)<sup>7</sup>, [Bozec A](#)<sup>8</sup>, [Gaggioli C](#)<sup>9</sup>.

**Regulation of CAF : epigenetic and mechanism (30480453)**

**Epigenetic regulation**

[Abrogation of myofibroblast activities in metastasis and fibrosis by methyltransferase inhibition.](#) Sala L, Franco-Valls H, Stanisavljevic J, Curto J, Vergés J, Peña R, Duch P, Alcaraz J, García de Herreros A, Baulida J. *Int J Cancer*. 2019 Apr 29. doi: 10.1002/ijc.32376. [Epub ahead of print] PMID: 31032902

[Proteomics reveals NNMT as a master metabolic regulator of cancer-associated fibroblasts.](#) Eckert MA, Coscia F, Chryplewicz A, Chang JW, Hernandez KM, Pan S, Tienda SM, Nahotko DA, Li G, Blaženović I, Lastra RR, Curtis M, Yamada SD, Perets R, McGregor SM, Andrade J, Fiehn O, Moellering RE, Mann M, Lengyel E. *Nature*. 2019 May;569(7758):723-728. doi: 10.1038/s41586-019-1173-8. Epub 2019 May 1. PMID:31043742

[Cancer-associated fibroblasts promote prostate cancer malignancy via metabolic rewiring and mitochondrial transfer.](#) Ippolito L, Morandi A, Taddei ML, Parri M, Comito G, Iscaro A, Raspollini MR, Magherini F, Rapizzi E, Masquelier J, Muccioli GG, Sonveaux P, Chiarugi P, Giannoni E. *Oncogene*. 2019 Apr 1. doi: 10.1038/s41388-019-0805-7. [Epub ahead of print] PMID:30936458

[Cancer-associated fibroblasts induce cancer cell apoptosis that regulates invasion mode of tumours.](#) Itoh G, Chida S, Yanagihara K, Yashiro M, Aiba N, Tanaka M. *Oncogene*. 2017 Aug; 36(31):4434-4444. doi: 10.1038/onc.2017.49. Epub 2017 Apr 3. PMID:28368418

[Cancer-associated fibroblasts induce metalloprotease-independent cancer cell invasion of the basement membrane.](#) **Glentis A**, Oertle P, Mariani P, Chikina A, El Marjou F, Attieh Y, Zaccarini F, Lae M, Loew D, Dingli F, Sirven P, Schoumacher M, Gurchenkov BG, Plodinec

M, Vignjevic DM. Nat Commun. 2017 Oct 13;8(1):924. doi: 10.1038/s41467-017-00985-8. Erratum in: [Nat Commun. 2018 Mar 7;9\(1\):1036](#). PMID:29030636

[Cancer-associated fibroblast-derived annexin A6+ extracellular vesicles support pancreatic cancer aggressiveness.](#) Leca J, Martinez S, Lac S, Nigri J, Secq V, Rubis M, Bressy C, Serge A, Lavaut MN, Dusetti N, Loncle C, Roques J, Pietrasz D, Bousquet C, Garcia S, Granjeaud S, Ouaisi M, Bachet JB, Brun C, Iovanna JL, Zimmermann P, Vasseur S, Tomasini R. J Clin Invest. **2016** Nov 1;126(11):4140-4156. doi: 10.1172/JCI87734. Epub **2016** Oct 4. PMID: 27701147

CAF and MEC

MAX 10 pages

Key points:

(3-5 key points/sentences that summarize your article)

• • •

## Brouillon

it creates an ecosystem allowing tumors cell to proliferate and to invade.headings)

Emerging functions

[A subtype of cancer-associated fibroblasts with lower expression of alpha-smooth muscle actin suppresses stemness through BMP4 in oral carcinoma.](#)

Patel AK, Vipparthi K, Thatikonda V, Arun I, Bhattacharjee S, Sharan R, Arun P, Singh S. *Oncogenesis*. 2018 Oct 5;7(10):78. doi: 10.1038/s41389-018-0087-x. PMID:30287850

CAF are activated fibroblasts called myofibroblasts (MF) that synthesize ECM components like fibroblasts, in surrounding stroma and display features of smooth muscle cells by expressing alpha-smooth muscle actin (a-SMA) coded par *ACTA* gene, conferring higher contractility to MF than fibroblasts. A well-known activator of a-SMA promoter is smad3, which is part of TGFB-dependent signaling pathway. In CCA, a genomic signature of TGFB has been identified in stromal compartment. Even if the soluble mediators secreted by pancreatic tumor cells have not be identified, one may hypothesize that TGF-b (transforming growth factor beta), a major profibrogenic cytokines, is one of them {Fabregat, 2018 #8410}.

[Mesenchymal Stem Cells are Recruited and Activated into Carcinoma-Associated Fibroblasts by Prostate Cancer Microenvironment-Derived TGF- \$\beta\$ 1.](#) Barcellos-de-Souza P, Comito G, Pons-Segura C, Taddei ML, Gori V, Becherucci V, Bambi F, Margheri F, Laurenzana A, Del Rosso M, Chiarugi P. *Stem Cells*. 2016 Oct;34(10):2536-2547. doi: 10.1002/stem.2412. Epub 2016 Jul 4. PMID:27300750

[Phenotypic and functional heterogeneity of cancer-associated fibroblast within the tumor microenvironment.](#) Ishii G et al. *Adv Drug Deliv Rev*. (2016)

Les CAF du CCA expriment le VEGFA et C (Cadamuro J *hepatol* 30553841

PDGF-D stimulates VEGF-C and VEGF-A production by fibroblasts, resulting in expansion of the lymphatic vasculature and tumor cell intravasation. This critical process in the early metastasis of cholangiocarcinoma may be blocked by inducing CAF apoptosis or by inhibiting the PDGF-D-induced axis.



**Acknowledgements**

LF and JV are member of the European Network for the Study of Cholangiocarcinoma (ENSCCA) and participates in the initiative COS action EURO-CHOLANGIO-NET granted by the COST Association (CA18122).

**Conflicts of interest**

None.

**Figure legends:**

(Attach figures and tables separately) Fig 1.

Heading:

Legend:

Source (when reusing previously published figures please ensure they are the same as the original and not adapted):

**References and recommended reading:**

Papers of particular interest, published within the annual period of review, (18 months/ 2012-2013) have been highlighted as:

- of special interest
- of outstanding interest

(1) Author, A, Author, B, Author, C et al; Title of reference; Publication name; Year; Volume; Issue; Page numbers

- an interesting insight into...

(2) (3) ...

## References

At least 80% of the references in a review should be from the period reviewed (published in the past year to 18 months). Most of these should have one or two bullets and annotations.

### 1. Bullets and Annotations

The important references from the period reviewed must have one or two bullets and an annotation. These are a key feature of Current Opinion journals.

- 
- Figures and tables should not be embedded within the text but should be submitted as separate files.

• Figures should be in JPEG, TIFF, EPS, PPT or WORD formats and should
--

have a resolution of at least 300 dpi to be suitable for printing. Please see <a href="#">Creating Digital Artwork (PDF)</a> for the full digital artwork requirements.
---

#### Bulleted references must

- • Have been published during the period reviewed by the issue (during the past year).
- • Have one bullet (\*) for special interest and two bullets (\*\*) for outstanding interest.
- • Be annotated with a brief description of the paper's importance.

When referencing and annotating your own work from previously published material you are limited to one bullet point.

**Note:** Any annotations on references from outside of the review period will be removed unless a justification is submitted to the journal office.

An example of bulleted and annotated reference section is shown below.

#### One bullet annotations:

\* Seror R, Sordet C, Guillevin L, et al. Tolerance and efficacy of rituximab and changes in serum B cell biomarkers in patients with systemic complications of primary Sjögren's syndrome. *Ann Rheum Dis* 2007; 66:351–357.

This is the first clinical trial to demonstrate the efficacy of B-cell depletion in SjS. This article highlights the importance of B cells in the pathogenesis of SjS.

#### Two bullet annotations:

\*\* Lavie F, Miceli-Richard C, Ittah M, et al. Increase of B-cell activating factor of the TNF family (BAFF) after rituximab: insights into a new regulating system of BAFF production. *Ann Rheum Dis* 2007; 66:700–703.

This study describes the elevation in BAFF levels that occurs in serum of patients who have been treated with B-cell depleting agents. This observation may have important consequences, following treatment, in promoting the corruption of B- cell tolerance and leading to disease relapse.