Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Systems Biology

Metabolic pathways fuelling protumourigenic cancer-associated fibroblast functions

Emily J. Kay[1] and Sara Zanivan[1,2]

Abstract

Cancer-associated fibroblasts (CAFs) play many roles in supporting tumour growth and progression, and metabolic rewiring is known to be a hallmark of CAF activation. How to effectively target CAF metabolism is still an open question, however. Recent research shows that CAFs and cancer cells engage in complex metabolic crosstalk, which may offer strategies to metabolically target both tumour and stroma. CAF metabolic rewiring also regulates intrinsic CAF protumourigenic functions, by inducing epigenetic changes to maintain CAF activation and by promoting hallmarks of CAFs such as extracellular matrix (ECM) production and immunosuppression. Finally, the emerging field of CAF subpopulations has opened up possibilities for metabolically targeting specific protumourigenic subgroups and raises new questions about how we define and target CAFs.

Addresses

[1] Cancer Research UK Beatson Institute, Glasgow G611BD, UK
[2] Institute of Cancer Sciences, University of Glasgow, Glasgow, G611QH, UK

Corresponding author: Zanivan, Sara (s.zanivan@beatson.gla.ac.uk)

Current Opinion in Systems Biology 2021, **28:**100377

This review comes from a themed issue on **Metabolic Networks (2022)**

Edited by **Sarah-Maria Fendt** and **Markus Ralser**

For complete overview of the section, please refer the article collection - Metabolic Networks (2022)

Available online 27 August 2021

https://doi.org/10.1016/j.coisb.2021.100377

Keywords

CAF, Metabolism, Cancer, Epigenetics, Crosstalk signalling, CAF subpopulations, Extracellular matrix, Tumour microenvironment.

Introduction

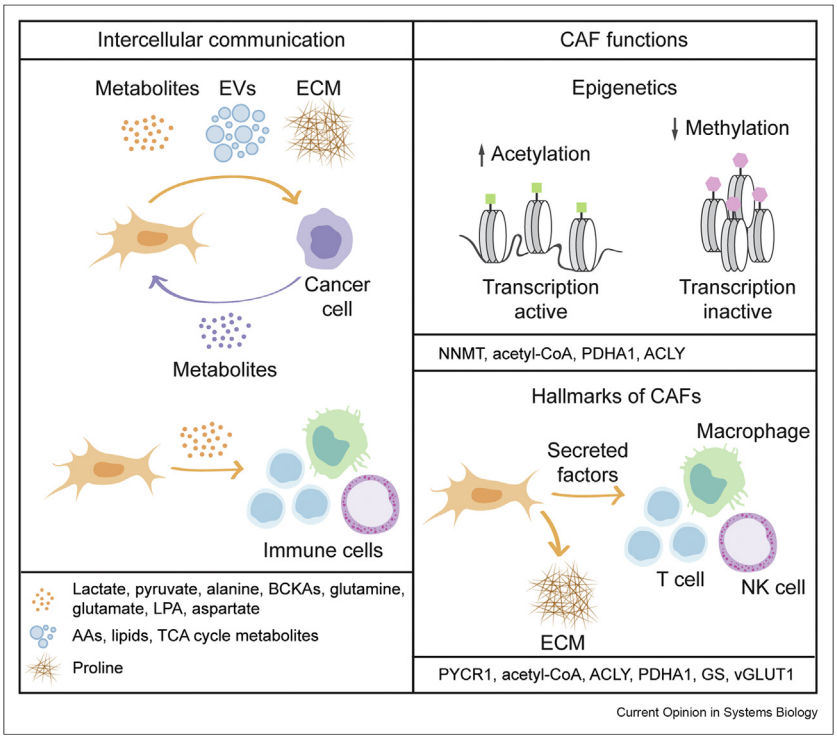
Cancer-associated fibroblasts (CAFs) are one of the most abundant cell types of the tumour microenvironment (TME) and are known to play a key role in all stages of tumour progression, displaying both tumour-promoting and tumour-restraining properties (reviewed in studies reported by Kalluri, Santi et al. and Sahai et al. [1–3]). As the importance of metabolic rewiring in tumour cells has become increasingly clear in recent years, in parallel, metabolic rewiring of CAFs has been shown to be both intertwined with that of tumour cells and to be a vital aspect of the protumourigenic CAF phenotype. Activated CAFs vastly increase production of many proteins that influence both tumour cells and other cells of the TME, including growth factors, cytokines, extracellular matrix components and proangiogenic factors. This review will discuss recent evidence that CAFs further influence the TME through increased secretion of metabolites and that CAFs also rewire their metabolism to support their protumourigenic functions (see Figures 1 and 2). We also give perspectives on the future of the field and how new technologies can enhance our understanding of CAF metabolism in the context of the tumour.

Cancer-associated fibroblast/cancer cell metabolic crosstalk

In the past decade, studies have shown that CAFs secrete metabolites which are taken up by tumour cells and used to support tumour progression. Initially, research focussed on the role of increased glycolysis and autophagy in CAFs, providing lactate, amino acids and ketone bodies to support tumour growth [4–7]. These studies provided initial evidence of metabolic crosstalk between CAFs and cancer cells and opened up the field of CAF metabolism for further investigation.

Increased lactate production owing to upregulation of glycolysis in CAFs is well-established; however, glycolysis-derived pyruvate is also emerging as a key protumourigenic metabolite. CAFs may be a major source of pyruvate in the TME. Pyruvate was secreted at high levels by CAFs derived from patients with breast cancer [8] and was also highly abundant in pancreatic ductal adenocarcinoma (PDAC) CAF-conditioned media [9]. Extracellular pyruvate maintained redox homoeostasis and enabled resistance to mitochondrial inhibitors in PDAC cells [9]. Pyruvate secreted by CAFs also promoted lymphoma cell survival [10], and extracellular pyruvate supported extracellular matrix (ECM) remodelling by metastatic breast cancer cells [11]. Therefore, CAF-secreted pyruvate has emerged as a key metabolite influencing several aspects of tumour progression.

Figure 1



Roles of CAF metabolism in cancer. This table highlights the roles described so far of CAF metabolism in cancer. Metabolites can be transferred to cancer cells and potentially (not yet shown) immune cells through their secretome, extracellular vesicles (EVs) and extracellular matrix proteins. CAF metabolism also controls intrinsic CAF functions via epigenetic regulation of histone modifications, regulation of signalling pathways and providing amino acids for extracellular matrix (ECM) production. After each section, there is a list of metabolites and enzymes that have been shown to contribute to the mechanisms represented in the drawing above. CAF, cancer-associated fibroblast.

Lipids have also emerged as protumourigenic CAF-secreted metabolites. Patient-derived CAFs from breast and colorectal cancers had increased fatty acid synthase (FASN) expression and produced more lipids, which were taken up by tumour cells and enhanced proliferation and metastasis [12,13]. CAF-derived lipids may be particularly relevant in pancreatic cancer in which CAFs are activated from resident pancreatic stellate cells (PSCs). Quiescent PSCs are rich in lipid droplets, but on activation, these are released. Auciello et al. [14] demonstrated that these lipids act as signalling molecules and fuel biomass production in pancreatic cancer cells and further discovered that PSCs are a major source of lysophosphatidic acid, activating phosphoinositide 3-kinase/RAC serine/threonine-protein kinase (PI3K/Akt) signalling in cancer cells.

Recent research has also highlighted the complexity of tumour cell-CAF crosstalk in rewiring CAF metabolism. The role of transforming growth factor beta (TGF-β) signalling and oxidative stress in stimulating glycolysis and autophagy in CAFs is well-documented [5,15]; however, it is becoming clear that cancer cells regulate CAF metabolism via a wide variety of mechanisms. TGF-β signalling was further shown to promote production and secretion of branched-chain keto acids in PDAC patient-derived CAFs via increased branched-chain-amino-acid aminotransferase (BCAT1) expression. Branched-chain keto acids were then used by tumour cells as a source of carbon and nitrogen [16]. Extracellular vesicles secreted by tumour cells are also known to mediate CAF-cancer cell crosstalk. Breast cancer-derived exosomes were shown to activate proto-oncogene protein MYC signalling in fibroblasts and increase both glucose and glutamine metabolism [17]. Equally, CAF-derived exosomes can be a source of metabolites to support cancer cell growth [18]. CAFs also depend on tumour cell-derived metabolites to support their metabolic needs, and increased glycolysis in CAFs can be stimulated by tumour cell-derived glycogen [19], or lysophosphatidic acid [20]. Furthermore, supporting the complexity of CAF/cancer cell metabolism is a study in ovarian cancer, which found that CAFs upregulate glutamine production and secretion [21]. Conversely, cancer cells secreted glutamate that was used by CAFs, creating a cycle that supports both CAFs and cancer cells. Inhibiting both glutamine and glutamate production reduced tumour growth *in vivo*, providing a good example of how cotargeting CAF and cancer cell metabolism can treat tumours more effectively.

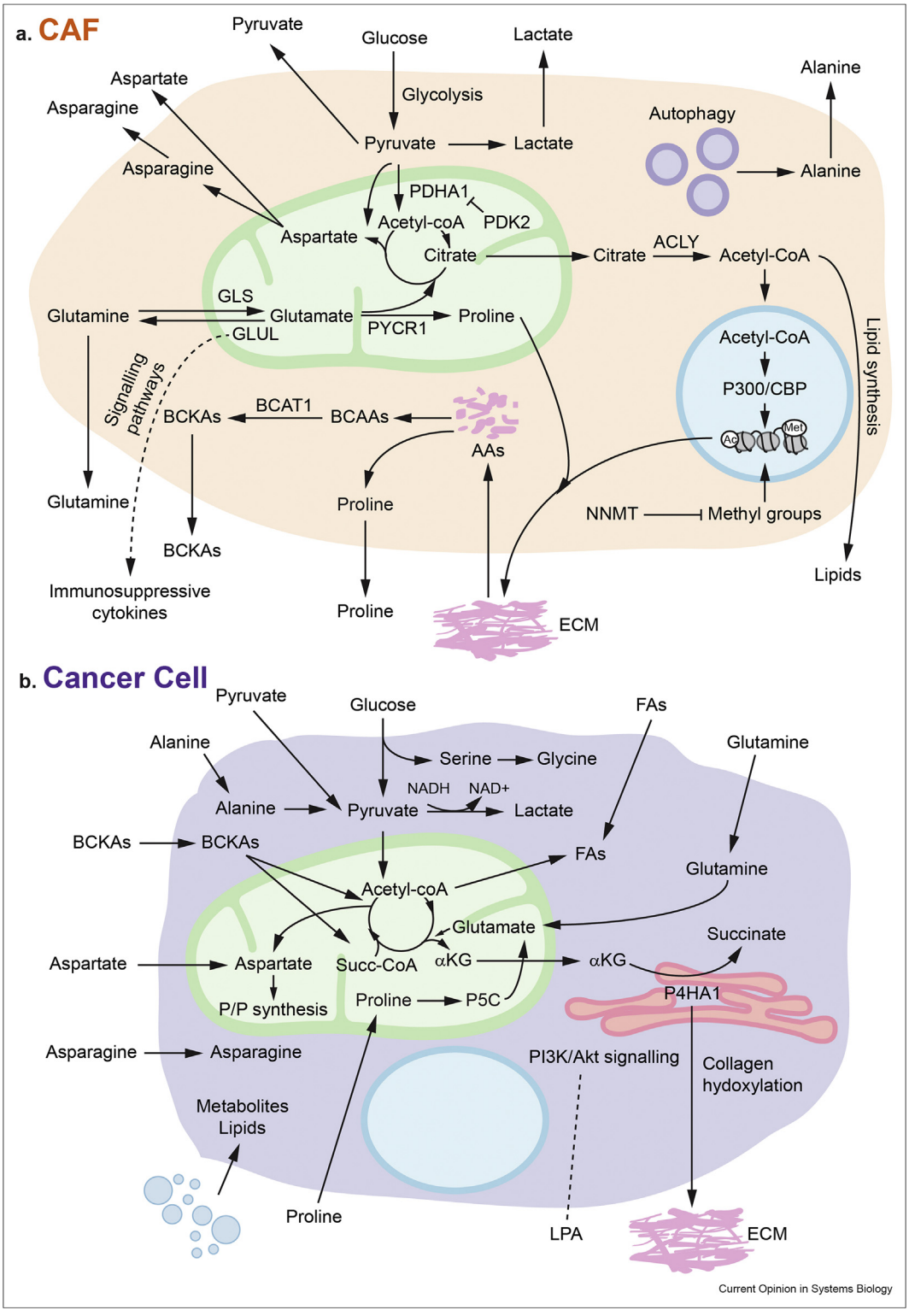
CAF metabolism is also regulated by other factors in the TME. The ECM is produced mostly by CAFs, but both CAFs and cancer cells influence ECM remodelling, crosslinking and stiffness. CAFs upregulated both glycolysis and oxidative phosphorylation on stiffer matrices. In addition, stiffer matrices induced a feedback loop between CAFs and cancer cells in which CAFs secreted aspartate that was taken up by cancer cells, and cancer cells secreted glutamate that was used by CAFs. This metabolic loop promoted tumour growth and invasion, and, crucially, could be disrupted by inhibiting glutaminase (GLS1) in both CAFs and cancer cells, giving the possibility to simultaneously target tumour and stroma [22]. Nutrient deprivation is also a common feature of the TME, and prostate cancer-derived CAFs were shown to support tumours under glutamine deprivation by upregulation of the pyruvate carboxylase-asparagine synthase cascade via p62 downregulation and cyclic AMP-dependent transcription factor ATF4. Stromal asparagine was used as a replacement nitrogen source by both CAFs and cancer cells [23]. Therefore, CAF metabolic rewiring can be stimulated through a combination of tumour cell-derived growth factors, metabolites and vesicles, as well as stressed such as nutrient deprivation, reactive oxygen species (ROS) and ECM stiffness. Feedback loops in which cancer cells and CAFs metabolically support each other may provide ways to simultaneously target CAFs and cancer cells.

Metabolism supports epigenetic changes and cancer-associated fibroblast hallmarks

Metabolic rewiring in CAFs can support other aspects of tumour development and the TME aside from providing nutrients to tumour cells. First, CAFs also rewire their metabolism to support their own phenotypic changes on activation. CAFs need to alter their epigenetic profile to maintain expression of genes involved in their protumourigenic phenotype. Nicotinamide N-methyltransferase (NNMT), which catalyses transfer of a methyl group from S-adenosyl methionine to nicotinamide, is upregulated in the stroma of ovarian, colorectal and gastric cancers [24–26] and associated with poor prognosis. NNMT reduced histone methylation in ovarian CAFs, and this led to widespread alterations in gene expression, including expression of CAF markers, tumour-promoting cytokines and ECM components [24]. Hypomethylation of promoters of glycolytic genes has also been observed in breast CAFs subjected to hypoxia [8]. Hypoxic signalling via hypoxia-inducible factor 1-alpha (HIF1-α) has previously been shown to induce a more glycolytic and autophagic CAF phenotype, supporting tumour cell metabolism via secretion of lactate and other metabolites [15,27]. Interestingly, the methylation pattern remained even when oxygen levels were restored, suggesting that stimuli in the TME can support an epigenetic shift in CAFs that enables them to maintain their protumourigenic phenotype even when those factors are removed. Decreased histone methylation often occurs with a corresponding increase in acetylation, and a study showed that breast CAFs have overall increased histone acetylation compared with normal fibroblasts (Kay et al., BioRxiv, doi: 10.1101/2020.05.30.125237). Histone acetylation is supported by the metabolite acetyl-CoA, and the study further uncovered that CAFs upregulate acetyl-CoA production through pyruvate dehydrogenase (PDH) activation and that this supports histone acetylation and expression of collagens. Overall, research points to CAFs metabolically inducing an epigenetic shift towards transcription activation, consistent with their increased output of growth factors, ECM components and proinflammatory cytokines.

In addition to epigenetic rewiring, CAF metabolism can support further hallmarks of their activated phenotype. Collagen makes up a large proportion of the stromal ECM (role of the ECM in cancer reviewed in studies reported by Cox, Kai et al and Winkler et al. [28–30]). Collagen itself is also a means of metabolic crosstalk between CAFs and cancer cells because PDAC cancer cells can take up and degrade collagen as a source of amino acids, and especially a source of proline [31]. Collagen proteins contain unusually high levels of both glycine and proline, and therefore, their translation places unique metabolic pressures on CAFs to produce these amino acids. CAFs from patients with breast cancer upregulated proline production via pyrroline-5-carboxylate reductase 1 (PYCR1), which was necessary to support collagen production. Stromal PYCR1 knockdown additionally reduced tumour growth and metastasis *in vivo* (Kay et al., BioRxiv, doi: 10.1101/2020.05.30.125237). Upregulated production of both proline and glycine to support collagen production was also observed in TGF-β-activated lung fibroblasts [32,33]. Immunosuppression is another hallmark of CAF activation. Netrin G1 (NetG1)+ CAFs from PDAC were shown to upregulate glutamine and glutamate synthesis, which were taken up and used as fuel by cancer cells. Interestingly, NetG1 also promoted immunosuppressive cytokine production by CAFs. Clustered regularly interspaced short palindromic repeats (CRISPR)-mediated knockout of either NetG1, glutamine synthetase (GS) or vesicular glutamate transporter 1 (VGlut1) reduced cytokine production and enabled natural killet (NK) cell-induced death of PDAC cells [34]. Although the role of CAF-secreted lactate is well-established as a fuel for tumour cells, lactate is also an immunosuppressive metabolite. Lactate promotes protumourigenic M2 macrophage polarisation, inhibits NK cell activity and reduces T cell infiltration in tumours [35–37]. Therefore, targeting CAF metabolism can not only affect cancer cells but also regulate other aspects of the TME that impacts tumour progression.

Figure 2



Metabolic crosstalk between CAF sand other tumour cells. (a). Summary of the pathways and metabolites regulating the communication between CAFs and other cell types and support hallmarks of CAFs. **(b)**. Summary of the pathways and metabolites regulated by CAFs in the cancer cells. CAF, cancer-associated fibroblast.

Cancer-associated fibroblast plasticity and subpopulations

A further layer of complexity to the CAF metabolic phenotype is the discovery of different CAF subpopulations, which have varied roles in the TME. The most commonly characterised subpopulations are the myofibroblastic CAFs (myofibroblastic, contractile, ECM producing) and the inflammatory CAFs, (inflammatory, cytokine-producing). These were first identified in PDAC, but similar populations have subsequently been found in other solid tumours including breast, ovarian and lung [38–40]. Further research has uncovered multiple subpopulations, including the antigen-presenting CAFs in PDAC [41] and up to eight populations in breast cancer [40]. As yet, little research has been performed into the metabolic phenotypes of these CAFs. Interestingly, expression of genes in the proline synthesis pathway is upregulated in myofibroblastic CAFs more than inflammatory CAFs, which supports proline availability being important for CAF ECM production (Kay et al., BioRxiv, doi: 10.1101/2020.05.30.125237). This suggests that the metabolic status of CAF subpopulations can support their function. In PDAC, a novel CAF subpopulation with a highly active metabolic state (MeCAF) has recently been described [42]. MeCAF abundance correlated with poorer prognosis but better response to immunotherapy, suggesting that the metabolism of CAF subpopulations could be used to inform on therapeutic strategy. In addition, different subpopulations may be more or less prevalent in different types of tumours, as has already been shown in subtypes of breast cancer [43]. CAF metabolism, as determined by levels of glycolysis and oxidative phosphorylation (OXPHOS), has also been shown to differ between different breast cancer subtypes, and it will be interesting to determine whether this mirrors the presence of different CAF subpopulations [44]. The discovery of CAF subpopulations raises the question of whether it is possible to metabolically target specific subpopulations, and if so, which subpopulation(s) would have the most impact. In addition, CAF subpopulations appear to be plastic and able to differentiate between different subtypes depending on their position in the tumour and other context-dependent stimuli such as cytokine levels [38,40,45]. It seems likely that CAFs do not exist as clearly defined subgroups but instead exist across a spectrum of phenotypes. Given that the CAF phenotype can be regulated metabolically at the epigenetic level, it will be interesting to uncover whether targeting CAF metabolism can induce CAFs to differentiate between different subpopulations.

Future directions

Our understanding of the metabolic changes in CAFs and how this impacts tumour progression has expanded rapidly in recent years. However, there are still many questions to be addressed. It is becoming clear that the metabolic phenotypes of CAFs, cancer cells and other cells in the TME are closely intertwined and just as cancer cells depend on CAFs for nutritional support, so do CAFs rely on metabolites and factors produced by cancer cells to support their metabolism. Therefore, the most effective metabolic targets to target the tumour as a whole organ rather than focussing on only the tumour cells or cells of the TME are yet to be determined. Pathways such as proline or glutamine metabolism, which are upregulated in both CAFs and cancer cells and have protumourigenic functions in both, may be an efficient method of targeting several aspects of the TME. Alternatively, it may be necessary to target more than one pathway to disrupt metabolic crosstalk loops between CAFs and cancer cells. There are several emerging methods to further investigate metabolic crosstalk between CAFs and cancer cells in *in vivo* and *in vitro* systems. For example, tracing labelled metabolites into stable molecules such as proteins can eliminate the problems of rapid metabolite transfer between cell types and metabolite loss during cell sorting from tumours or three-dimensional coculture systems [46]. The recently developed flow cytometry-based method SCENITH (single-cell energetic metabolism by profiling translation inhibition) enables a seahorse-like analysis of mixed cell populations by inferring respiration, glycolysis and fatty acid oxidation (FAO) rates from changes in protein translation [47]. The growing field of mass spectrometry-based imaging, which enables spatial visualisation of metabolites and proteins within tissue sections [48], will also enable further investigation of the metabolic states of CAFs and cancer cells in the TME context.

The tools described previously can also be applied to uncover metabolic variations between CAF subpopulations. Recent and ongoing work into fibroblast heterogeneity and plasticity has opened up the possibility of targeting different subpopulations of CAFs within the TME, and it will be important to also distinguish the different metabolic phenotypes of these CAFs to determine the best therapeutic strategies for targeting cancer metabolism. Alpha smooth muscle actin (αSMA)-positive CAFs have previously been the main focus for targeting the stroma, but the emerging field of CAF subpopulations may uncover new definitions and markers for CAFs, and it remains to be determined whether CAF metabolic heterogeneity will play a role in this. Furthermore, it is becoming clear that CAFs can have both protumourigenic and antitumourigenic properties, particularly in PDAC in which stromal depletion enables tumour expansion [49,50]. Therefore, a good strategy to target CAFs may be to promote differentiation to a less tumour-promoting 'subpopulation', and targeting CAF metabolism could be a means to do this, potentially through metabolically altering the epigenetic profile of CAFs.

Conflict of interest statement

Nothing declared.

Acknowledgements

This work was funded by Cancer Research UK (CRUK Beatson Institute A31287, CRUK Glasgow Centre A18076, CRUK A29800 to S.Z.) and Breast Cancer Now (2019AugPR1307 to S.Z.)

References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. Kalluri R: **The biology and function of fibroblasts in cancer**. *Nat Rev Canc* 2016, **16**:582–598.

2. Santi A, Kugeratski FG, Zanivan S: **Cancer associated fibroblasts: the architects of stroma remodeling**. *Proteomics* 2018, **18**, e1700167.

3. Sahai E, Astsaturov I, Cukierman E, DeNardo DG, Egeblad M, Evans RM, Fearon D, Greten FR, Hingorani SR, Hunter T, Hynes RO, Jain RK, Janowitz T, Jorgensen C, Kimmelman AC, Kolonin MG, Maki RG, Powers RS, Pure E, Ramirez DC, Scherz-Shouval R, Sherman MH, Stewart S, Tlsty TD, Tuveson DA, Watt FM, Weaver V, Weeraratna AT, Werb Z: **A framework for advancing our understanding of cancer-associated fibroblasts**. *Nat Rev Canc* 2020, **20**:174–186.

4. Sousa CM, Biancur DE, Wang X, Halbrook CJ, Sherman MH, Zhang L, Kremer D, Hwang RF, Witkiewicz AK, Ying H, Asara JM, Evans RM, Cantley LC, Lyssiotis CA, Kimmelman AC: **Pancreatic stellate cells support tumour metabolism through autophagic alanine secretion**. *Nature* 2016, **536**:479–483.

5. Guido C, Whitaker-Menezes D, Capparelli C, Balliet R, Lin Z, Pestell RG, Howell A, Aquila S, Ando S, Martinez-Outschoorn U, Sotgia F, Lisanti MP: **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**. *Cell Cycle* 2012, **11**:3019–3035.

6. Pavlides S, Whitaker-Menezes D, Castello-Cros R, Flomenberg N, Witkiewicz AK, Frank PG, Casimiro MC, Wang C, Fortina P, Addya S, Pestell RG, Martinez-Outschoorn UE, Sotgia F, Lisanti MP: **The reverse Warburg effect: aerobic glycolysis in cancer associated fibroblasts and the tumor stroma**. *Cell Cycle* 2009, **8**:3984–4001.

7. Whitaker-Menezes D, Martinez-Outschoorn UE, Lin Z, Ertel A, Flomenberg N, Witkiewicz AK, Birbe RC, Howell A, Pavlides S, Gandara R, Pestell RG, Sotgia F, Philp NJ, Lisanti MP: **Evidence for a stromal-epithelial "lactate shuttle" in human tumors: MCT4 is a marker of oxidative stress in cancer-associated fibroblasts**. *Cell Cycle* 2011, **10**:1772–1783.

8. Becker LM, O'Connell JT, Vo AP, Cain MP, Tampe D, Bizarro L, Sugimoto H, McGow AK, Asara JM, Lovisa S, McAndrews KM, Zielinski R, Lorenzi PL, Zeisberg M, Raza S, LeBleu VS, Kalluri R: **Epigenetic reprogramming of cancer-associated fibroblasts deregulates glucose metabolism and facilitates progression of breast cancer**. *Cell Rep* 2020, **31**:107701.

9. * Kerk LL Samuel A, Myers Amy L, Chen Brandon, Peter Sajjakulnukit, Robinson Anthony, Thurston Galloway, Nelson Barbara S, Kemp Samantha B, Steele Nina G, Hoffman Megan T, Wen Hui-Ju, Long Daniel, Ackenhusen Sarah E, Ramos Johanna, Gao Xiaohua, Zhang Li, Anthony Andren, Nwosu Zeribe C, Galbán Stefanie, Halbrook Christopher J, Lombard David B, Ying Haoqiang, Crawford Howard C, di Magliano Marina Pasca, Shah Yatrik M, Lyssiotis Costas A: *The pancreatic tumor microenvironment buffers redox imbalance imposed by disrupted mitochondrial metabolism*. bioRxiv; 2020.
Pyruvate is emerging as a key CAF secreted metabolite and influences multiple aspects of tumour progression aside from fuelling tumour growth. In this work, CAF derived pyruvate maintained redox homeostasis and enabled resistance to mitochondrial inhibitors in PDAC cells.

10. Sakamoto A, Kunou S, Shimada K, Tsunoda M, Aoki T, Iriyama C, Tomita A, Nakamura S, Hayakawa F, Kiyoi H: **Pyruvate secreted from patient-derived cancer-associated fibroblasts supports survival of primary lymphoma cells**. *Canc Sci* 2019, **110**:269–278.

11. Elia I, Rossi M, Stegen S, Broekaert D, Doglioni G, van Gorsel M, Boon R, Escalona-Noguero C, Torrekens S, Verfaillie C, Verbeken E, Carmeliet G, Fendt SM: **Breast cancer cells rely on environmental pyruvate to shape the metastatic niche**. *Nature* 2019, **568**:117–121.

12. Lopes-Coelho F, Andre S, Felix A, Serpa J: **Breast cancer metabolic cross-talk: fibroblasts are hubs and breast cancer cells are gatherers of lipids**. *Mol Cell Endocrinol* 2018, **462**:93–106.

13. Gong J, Lin Y, Zhang H, Liu C, Cheng Z, Yang X, Zhang J, Xiao Y, Sang N, Qian X, Wang L, Cen X, Du X, Zhao Y: **Reprogramming of lipid metabolism in cancer-associated fibroblasts potentiates migration of colorectal cancer cells**. *Cell Death Dis* 2020, **11**:267.

14. Auciello FR, Bulusu V, Oon C, Tait-Mulder J, Berry M, Bhattacharyya S, Tumanov S, Allen-Petersen BL, Link J, Kendsersky ND, Vringer E, Schug M, Novo D, Hwang RF, Evans RM, Nixon C, Dorrell C, Morton JP, Norman JC, Sears RC, Kamphorst JJ, Sherman MH: **A stromal lysolipid-autotaxin signaling Axis promotes pancreatic tumor progression**. *Canc Discov* 2019, **9**:617–627.

15. Martinez-Outschoorn UE, Trimmer C, Lin Z, Whitaker-Menezes D, Chiavarina B, Zhou J, Wang C, Pavlides S, Martinez-Cantarin MP, Capozza F, Witkiewicz AK, Flomenberg N, Howell A, Pestell RG, Caro J, Lisanti MP, Sotgia F: **Autophagy in cancer associated fibroblasts promotes tumor cell survival: role of hypoxia, HIF1 induction and NFkappaB activation in the tumor stromal microenvironment**. *Cell Cycle* 2010, **9**:3515–3533.

16. Zhu Z, Achreja A, Meurs N, Animasahun O, Owen S, Mittal A, Parikh P, Lo TW, Franco-Barraza J, Shi J, Gunchick V, Sherman MH, Cukierman E, Pickering AM, Maitra A, Sahai V, Morgan MA, Nagrath S, Lawrence TS, Nagrath D: **Tumour-reprogrammed stromal BCAT1 fuels branched-chain ketoacid dependency in stromal-rich PDAC tumours**. *Nat Metab* 2020, **2**:775–792.

17. Yan W, Wu X, Zhou W, Fong MY, Cao M, Liu J, Liu X, Chen CH, Fadare O, Pizzo DP, Wu J, Liu L, Liu X, Chin AR, Ren X, Chen Y, Locasale JW, Wang SE: **Cancer-cell-secreted exosomal miR-105 promotes tumour growth through the MYC-dependent metabolic reprogramming of stromal cells**. *Nat Cell Biol* 2018, **20**:597–609.

18. Zhao H, Yang L, Baddour J, Achreja A, Bernard V, Moss T, Marini JC, Tudawe T, Seviour EG, San Lucas FA, Alvarez H, Gupta S, Maiti SN, Cooper L, Peehl D, Ram PT, Maitra A, Nagrath D: **Tumor microenvironment derived exosomes pleiotropically modulate cancer cell metabolism**. *Elife* 2016, **5**, e10250.

19. Curtis M, Kenny HA, Ashcroft B, Mukherjee A, Johnson A, Zhang Y, Helou Y, Batlle R, Liu X, Gutierrez N, Gao X, Yamada SD, Lastra R, Montag A, Ahsan N, Locasale JW, Salomon AR, Nebreda AR, Lengyel E: **Fibroblasts mobilize tumor cell glycogen to promote proliferation and metastasis**. *Cell Metabol* 2019, **29**:141–155 e9.

20. Radhakrishnan R, Ha JH, Jayaraman M, Liu J, Moxley KM, Isidoro C, Sood AK, Song YS, Dhanasekaran DN: **Ovarian cancer cell-derived lysophosphatidic acid induces glycolytic shift and cancer-associated fibroblast-phenotype in normal and peritumoral fibroblasts**. *Canc Lett* 2019, **442**:464–474.

21. Yang L, Achreja A, Yeung TL, Mangala LS, Jiang D, Han C, Baddour J, Marini JC, Ni J, Nakahara R, Wahlig S, Chiba L, Kim SH, Morse J, Pradeep S, Nagaraja AS, Haemmerle M, Kyunghee N, Derichsweiler M, Plackemeier T, Mercado-Uribe I, Lopez-Berestein G, Moss T, Ram PT, Liu J, Lu X, Mok SC, Sood AK, Nagrath D: **Targeting stromal glutamine synthetase in tumors disrupts tumor microenvironment-regulated cancer cell growth**. *Cell Metabol* 2016, **24**:685–700.

22. ** 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: **Tumor-stroma mechanics coordinate amino acid availability to sustain tumor growth and malignancy**. *Cell Metabol* 2019, **29**: 124–140 e10.

This work demonstrates the co-dependency of CAF and cancer cell metabolism via an aspartate-glutamate loop, and highlights the importance of other factors in the TME, in this case ECM stiffness, in regulating CAF/cancer cell metabolic crosstalk.

23. Linares JF, Cordes T, Duran A, Reina-Campos M, Valencia T, Ahn CS, Castilla EA, Moscat J, Metallo CM, Diaz-Meco MT: **ATF4-Induced metabolic reprograming is a synthetic vulnerability of the p62-deficient tumor stroma**. *Cell Metabol* 2017, **26**:817–829 e6.

24. ** 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: **Proteomics reveals NNMT as a master metabolic regulator of cancer-associated fibroblasts**. *Nature* 2019, **569**:723–728.

CAF metabolic rewiring regulates the CAF epigenetic phenotype by decreasing histone methylation, leading to increased active transcription. This induces expression of key genes involved in CAF activation.

25. Song M, Li Y, Miao M, Zhang F, Yuan H, Cao F, Chang W, Shi H, Song C: **High stromal nicotinamide N-methyltransferase (NNMT) indicates poor prognosis in colorectal cancer**. *Cancer Med* 2020, **9**:2030–2038.

26. Zhang L, Song M, Zhang F, Yuan H, Chang W, Yu G, Niu Y: **Accumulation of nicotinamide N-methyltransferase (NNMT) in cancer-associated fibroblasts: a potential prognostic and predictive biomarker for gastric carcinoma**. *J Histochem Cytochem* 2021, **69**:165–176.

27. Zhang D, Wang Y, Shi Z, Liu J, Sun P, Hou X, Zhang J, Zhao S, Zhou BP, Mi J: **Metabolic reprogramming of cancer-associated fibroblasts by IDH3alpha downregulation**. *Cell Rep* 2015, **10**:1335–1348.

28. Cox TR: **The matrix in cancer**. *Nat Rev Canc* 2021, **21**:217–238.

29. Kai F, Drain AP, Weaver VM: **The extracellular matrix modulates the metastatic journey**. *Dev Cell* 2019, **49**:332–346.

30. Winkler J, Abisoye-Ogunniyan A, Metcalf KJ, Werb Z: **Concepts of extracellular matrix remodelling in tumour progression and metastasis**. *Nat Commun* 2020, **11**:5120.

31. Olivares O, Mayers JR, Gouirand V, Torrence ME, Gicquel T, Borge L, Lac S, Roques J, Lavaut MN, Berthezene P, Rubis M, Secq V, Garcia S, Moutardier V, Lombardo D, Iovanna JL, Tomasini R, Guillaumond F, Vander Heiden MG, Vasseur S: **Collagen-derived proline promotes pancreatic ductal adenocarcinoma cell survival under nutrient limited conditions**. *Nat Commun* 2017, **8**:16031.

32. Schworer S, Berisa M, Violante S, Qin W, Zhu J, Hendrickson RC, Cross JR, Thompson CB: **Proline biosynthesis is a vent for TGFbeta-induced mitochondrial redox stress**. *EMBO J* 2020, **39**, e103334.

33. Nigdelioglu R, Hamanaka RB, Meliton AY, O'Leary E, Witt LJ, Cho T, Sun K, Bonham C, Wu D, Woods PS, Husain AN, Wolfgeher D, Dulin NO, Chandel NS, Mutlu GM: **Transforming growth factor (TGF)-beta promotes de Novo serine synthesis for collagen production**. *J Biol Chem* 2016, **291**: 27239–27251.

34. * Francescone R, Barbosa Vendramini-Costa D, Franco-Barraza J, Wagner J, Muir A, Lau AN, Gabitova L, Pazina T, Gupta S, Luong T, Rollins D, Malik R, Thapa RJ, Restifo D, Zhou Y, Cai KQ, Hensley HH, Tan Y, Kruger WD, Devarajan K, Balachandran S, Klein-Szanto AJ, Wang H, El-Deiry WS, Vander Heiden MG, Peri S, Campbell KS, Astsaturov I, Cukierman E: **Netrin G1 promotes pancreatic tumorigenesis through cancer-associated fibroblast-driven nutritional support and immunosuppression**. *Canc Discov* 2021, **11**:446–479.

Netrin G1+ CAFs upregulate glutamine metabolism to metabolically support cancer cells. This pathway also supports cytokine production, demonstrating that CAF metabolic rewiring can also influence other cells of the TME.

35. Colegio OR, Chu NQ, Szabo AL, Chu T, Rhebergen AM, Jairam V, Cyrus N, Brokowski CE, Eisenbarth SC, Phillips GM, Cline GW, Phillips AJ, Medzhitov R: **Functional polarization of tumour-associated macrophages by tumour-derived lactic acid**. *Nature* 2014, **513**:559–563.

36. Husain Z, Huang Y, Seth P, Sukhatme VP: **Tumor-derived lactate modifies antitumor immune response: effect on myeloid-derived suppressor cells and NK cells**. *J Immunol* 2013, **191**:1486–1495.

37. P.-N.A. Santos N, Baltazar F, Granja S: **Lactate as a regulator of cancer inflammation and immunity**. *Immunometabolism* 2019, **1**, e190015.

38. Ohlund D, Handly-Santana A, Biffi G, Elyada E, Almeida AS, Ponz-Sarvise M, Corbo V, Oni TE, Hearn SA, Lee EJ, Chio II , Hwang CI, Tiriac H, Baker LA, Engle DD, Feig C, Kultti A, Egeblad M, Fearon DT, Crawford JM, Clevers H, Park Y, Tuveson DA: **Distinct populations of inflammatory fibroblasts and myofibroblasts in pancreatic cancer**. *J Exp Med* 2017, **214**:579–596.

39. Chung HC, Cho EJ, Lee H, Kim WK, Oh JH, Kim SH, Lee D, Sung CO: **Integrated single-cell RNA sequencing analyses suggest developmental paths of cancer-associated fibroblasts with gene expression dynamics**. *Clin Transl Med* 2021, **11**:e487.

40. * Kieffer Y, Hocine HR, Gentric G, Pelon F, Bernard C, Bourachot B, Lameiras S, Albergante L, Bonneau C, Guyard A, Tarte K, Zinovyev A, Baulande S, Zalcman G, Vincent-Salomon A, Mechta-Grigoriou F: **Single-cell analysis reveals fibroblast clusters linked to immunotherapy resistance in cancer**. *Canc Discov* 2020, **10**:1330–1351.

Identification and analysis of CAF subpopulations in breast cancer subtypes demonstrates CAF heterogeneity between different tumour types. The work also provides evidence of CAF plasticity via crosstalk between CAFs and immune cells.

41. Elyada E, Bolisetty M, Laise P, Flynn WF, Courtois ET, Burkhart RA, Teinor JA, Belleau P, Biffi G, Lucito MS, Sivajothi S, Armstrong TD, Engle DD, Yu KH, Hao Y, Wolfgang CL, Park Y, Preall J, Jaffee EM, Califano A, Robson P, Tuveson DA: **Cross-species single-cell analysis of pancreatic ductal adenocarcinoma reveals antigen-presenting cancer-associated fibroblasts**. *Canc Discov* 2019, **9**:1102–1123.

42. Wang Y, Liang Y, Xu H, Zhang X, Mao T, Cui J, Yao J, Wang Y, Jiao F, Xiao X, Hu J, Xia Q, Zhang X, Wang X, Sun Y, Fu D, Shen L, Xu X, Xue J, Wang L: **Single-cell analysis of pancreatic ductal adenocarcinoma identifies a novel fibroblast subtype associated with poor prognosis but better immunotherapy response**. *Cell Discov* 2021, **7**:36.

43. Costa A, Kieffer Y, Scholer-Dahirel A, Pelon F, Bourachot B, Cardon M, Sirven P, Magagna I, Fuhrmann L, Bernard C, Bonneau C, Kondratova M, Kuperstein I, Zinovyev A, Givel AM, Parrini MC, Soumelis V, Vincent-Salomon A, Mechta-Grigoriou F: **Fibroblast heterogeneity and immunosuppressive environment in human breast cancer**. *Canc Cell* 2018, **33**:463–479 e10.

44. Choi J, Kim DH, Jung WH, Koo JS: **Metabolic interaction between cancer cells and stromal cells according to breast cancer molecular subtype**. *Breast Cancer Res* 2013, **15**:R78.

45. Biffi G, Oni TE, Spielman B, Hao Y, Elyada E, Park Y, Preall J, Tuveson DA: **IL1-Induced JAK/STAT signaling is antagonized by TGFbeta to shape CAF heterogeneity in pancreatic ductal adenocarcinoma**. *Canc Discov* 2019, **9**:282–301.

46. Lau AN, Li Z, Danai LV, Westermark AM, Darnell AM, Ferreira R, Gocheva V, Sivanand S, Lien EC, Sapp KM, Mayers JR, Biffi G, Chin CR, Davidson SM, Tuveson DA, Jacks T, Matheson NJ, Yilmaz O, Vander Heiden MG: **Dissecting cell-type-specific metabolism in pancreatic ductal adenocarcinoma**. *Elife* 2020, **9**.

47. Arguello RJ, Combes AJ, Char R, Gigan JP, Baaziz AI, Bousiquot E, Camosseto V, Samad B, Tsui J, Yan P, Boissonneau S, Figarella-Branger D, Gatti E, Tabouret E, Krummel MF, Pierre P: **SCENITH: a flow cytometry-based method to functionally profile energy metabolism with single-cell resolution**. *Cell Metabol* 2020, **32**:1063–1075 e7.

48. Buchberger AR, DeLaney K, Johnson J, Li L: **Mass spectrometry imaging: a review of emerging advancements and future insights**. *Anal Chem* 2018, **90**:240–265.

49. Ozdemir BC, Pentcheva-Hoang T, Carstens JL, Zheng X, Wu CC, Simpson TR, Laklai H, Sugimoto H, Kahlert C, Novitskiy SV, De Jesus-Acosta A, Sharma P, Heidari P, Mahmood U, Chin L, Moses HL, Weaver VM, Maitra A, Allison JP, LeBleu VS, Kalluri R: **Depletion of carcinoma-associated fibroblasts and fibrosis induces immunosuppression and accelerates pancreas cancer with reduced survival**. *Canc Cell* 2014, **25**: 719–734.

50. Chen Y, Kim J, Yang S, Wang H, Wu CJ, Sugimoto H, LeBleu VS, Kalluri R: **Type I collagen deletion in alphaSMA(+) myofibroblasts augments immune suppression and accelerates progression of pancreatic cancer**. *Canc Cell* 2021, **39**: 548–565 e6.