Metabolic Relationship Between Cancer-Associated Fibroblasts and Cancer Cells

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Keywords
Cancer-associated fibroblasts · CAF-derived exosomes · Glutamine metabolism · Hypoxia-inducible factor-1 · Reverse Warburg effect · miRNA · TGF-β · Alanine · Cav-1

Abbreviations

ATG16L1	Autophagy-related 16 like 1
BNIP3	BCL2/adenovirus E1B 19 kDa protein-interacting protein 3
BNIP3L	BCL2/adenovirus E1B 19 kDa protein-interacting protein 3-like
CAF	Cancer-associated fibroblast
Cav-1	Caveolin-1
CDE	CAF-derived exosomes
CTSB	Cathepsin B
EMT	Epithelial-mesenchymal transition
FASN	Fatty acid synthase
FH	Fumarase
HIF-1	Hypoxia-inducible factor-1
IκB	Inhibitor of NF-κB
IκBK	IκB kinase
LDHA	Lactate dehydrogenase A
LDHB	Lactate dehydrogenase B
MCT	Monocarboxylate transporter
miRNA	microRNA
mtROS	Mitochondrial ROS
NF	Normal fibroblasts
NFκB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NHE1	Sodium-hydrogen exchanger 1
PDAC	Pancreatic ductal adenocarcinoma
PGC-1α	PPARG coactivator 1 alpha
PKFM	6-Phosphofructokinase, muscle type
PKM2	Pyruvate kinase isozymes M1/M2
PSC	Pancreatic stellate cells
ROS	Reactive oxygen species
SDH	Succinate dehydrogenase
SIRT	Sirtuin
TCA	Tricarboxylic acid
TGF-β	Transforming growth factor-beta
TME	Tumor microenvironment
TP53INP1	Tumor protein p53-inducible nuclear protein 1
α-KG	α-Ketoglutarate
α-SMA	α-Smooth muscle actin

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Key Points

- Cancer-associated fibroblasts undergo the reverse Warburg effect and provide cancer cells with glycolytic metabolites.
- The interaction between cancer cells and CAFs helps cancer cells manage the Warburg effect.
- Loss of stromal Cav-1 is a biomarker of poor prognosis in breast cancers.
- Exogenous and endogenous miRNAs are crucial in the metabolic reprogramming of CAFs.
- CAF-derived exosomes (CDEs) can reprogram the metabolic pathway of cancer cells.
- CAF-derived lactate is crucial in prostate cancer metabolic transformation towards OXPHOS.
- CAFs can transfer functional mitochondria to prostate cancer cells.
- CAFs augment cancer's addiction to glutamine and its metabolically relevant consequences.
- Alanine secreted by pancreatic stellate cells supports tumor metabolism.
- CAFs act as lipid synthesis factories for colorectal cancer cells.

1 Introduction

Cancer-associated fibroblasts (CAFs), a major component of the tumor microenvironment (TME), play an important role in cancer initiation, progression, and metastasis. Recent findings have demonstrated that the TME not only provides physical support for cancer cells but also directs cell-to-cell interactions (in this case, the interaction between cancer cells and CAFs). As cancer progresses, the CAFs also coevolve, transitioning from an inactivated state to an activated state. The elucidation and understanding of the interaction between cancer cells and CAFs will pave the way for new cancer therapies [1–3].

The TME is a heterogeneous environment consisting of fibroblasts, tumor-associated macrophages, adipocytes, an extracellular matrix, and mesenchymal stem cells [4]. The exact composition of each stroma varies depending on cancer and tissue type. To add to this variation, there is heterogeneity even within the CAF population itself. Different CAFs express different markers and influence stromal pro-tumorigenic capacity and cancer progression in diverse ways [5, 6].

CAFs, unlike normal fibroblasts (NF), are not passive bystanders. They possess similar characteristics to myofibroblasts, the fibroblasts responsible for wound healing and chronic inflammation, such as the expression of α-smooth muscle actin (α-SMA) [7, 8]. Regarded in a similar light, cancer might be considered a wound that cannot be healed. CAFs can originate from the activation and differentiation of quiescent fibroblasts, bone marrow-derived mesenchymal stem cells, and epithelial and endothelial cells [9].

The interaction of the TME, specifically among CAFs with cancer cells, is incontrovertible. The effect of CAFs on cancer is dependent on cancer type and stage. The production and secretion of growth factors, chemokines, cytokines, metabolites, and extracellular matrix components aid in the recruitment of various cell types, such as pericytes and endothelial cells, facilitating angiogenesis and bestowing chemoresistant properties to the cancer cells. In this chapter, we discuss the properties and characteristics of CAFs, and their importance in cancer progression.

As mentioned in the chapter "Different Tumor Microenvironments Lead to Different Metabolic Phenotypes" [10], Hanahan and Weinberg [11] have identified six hallmark capabilities of cancer cells: (1) self-sufficiency in growth signals, (2) insensitivity to anti-growth signals, (3) evasion of apoptosis, (4) limitless replicative potential, (5) sustained angiogenesis, and (6) tissue invasion and metastasis. The exact mechanisms by which the TME can influence cancer and lead to the acquisition of those hallmark capabilities are not yet fully understood. However, there is growing evidence suggesting that the manipulation of signal transduction pathways in cancer cells, CAFs, and altered metabolic pathways may play a role in the transformation process [12–17].

2 CAFs Undergo the Reverse Warburg Effect and Provide Cancer Cells with Glycolytic Metabolites

As mentioned in previous chapters, cancer cells undergo a phenomenon known as the Warburg effect, an increase in aerobic glycolysis to produce ATP even in normoxic conditions (normoxia or normal oxygen levels) [18]. Warburg initially attributed this phenomenon to malfunctioning mitochondria forcing the cancer cells to rely on glycolysis for energy production. Pyruvate and lactate, the two end products of glycolysis, were believed to be secreted by the hypoxic core of the tumor through monocarboxylate transporters (MCT4) for the adjacent oxygenated cancer cells to take up (via MCT1) and utilize as substrates for the tricarboxylic acid (TCA) cycle [19–21].

Recent studies, however, have revolutionized the way scientists view the TME, especially the cross talk between CAFs and cancer cells and the effect of this cross talk on metabolism. The Warburg effect, a phenomenon initially believed to be limited to cancer cells, has also been observed in the fibroblasts surrounding the cancer cells. To distinguish this CAF-related phenomenon from its cancer cell-related counterpart, Pavlides et al. named it the reverse Warburg effect [17]. Caveolin-1 (Cav-1) is a transforming growth factor-beta (TGF-β) type I receptor kinase inhibitor, and the loss of Cav-1 expression causes a myofibroblastic phenotype. By using Cav-1(−/−) fibroblasts, Pavlides et al. induced myofibroblastic differentiation to mimic CAFs. With the use of proteomics, they identified 25 proteins that were overexpressed when Cav-1 was suppressed. Eight of those proteins were glycolytic enzymes (Table 1), including M2-type pyruvate kinase (PKM2) and lactate dehydrogenase A (LDHA) [17]. These two enzymes are known to play crucial roles in the Warburg effect [22, 23]. Additionally, two enzymes involved in oxidative stress, peroxiredoxin 1 and catalase, were overexpressed under normoxic conditions, which indicates an increase of reactive oxygen species (ROS) in Cav-1(−/−) fibroblasts. Hypoxia-inducible factor-1 (HIF-1) is a transcription factor that responds to low oxygen concentrations. Under high levels of ROS, HIF-1 is stabilized. Subsequently, HIF-1, a regulator of all glycolytic enzymes, as well as glucose transporters, GLUT1 and GLUT3, induces aerobic glycolysis [17, 23].

A similar study performed by Shan et al. provided further evidence to support the reverse Warburg effect hypothesis. In this study, pancreatic associated fibroblasts expressed elevated levels of the glycolytic enzymes LDHA and PKM2, as well as the MCT4 transporter responsible for lactate secretion. Additionally, they observed that when pancreatic cancer cells were exposed to CAF-conditioned media, they underwent enhanced aerobic activity, causing an observable enlargement of the mitochondria. Furthermore, pancreatic cancer cells significantly increased the expression of MCT1, fumarate hydratase (FH), and succinate dehydrogenase (SDH). The overexpression of these enzymes further indicated the existence of metabolic coupling between CAFs and cancer cells [24].

3 The Interaction Between Cancer Cells and CAFs Helps Cancer Cells Manage the Warburg Effect

Even though the extratumoral high lactate concentration produced by CAFs is crucial for the progression of cancer, high intracellular lactate concentration causes a dramatic drop in the pH, which, if left untreated, results in the death of the cell. Interestingly, experimental research revealed a few mechanisms by which cancer cells manage the elevated lactate level as a result of the Warburg effect [25–28]. Cancer cells overexpress a Na^+/H^+ transporter, NHE1 (sodium-hydrogen exchanger 1), that pumps H^+ out of the cell and Na^+ into it, therefore neutralizing this decrease in pH caused by lactate [26]. Under hypoxic conditions, cancer cells overexpress carbonic anhydrase 9 (CA9), which is responsible for the conversion of carbon dioxide to bicarbonate to neutralize increased acidity [27]. Certain cancer cells also overexpress MCT4, the transporter involved in secreting lactate out of the cell. By doing this, if intracel-

Table 1 Glycolytic enzymes upregulated in Cav-1(−/−) mammary stromal fibroblasts

Glycolytic and metabolic enzymes	Metabolic reaction involved
M2-type pyruvate kinase	Phosphoenolpyruvate → pyruvate
Phosphoglycerate kinase I	Glycerate-1,3P2 ↔ glycerate-3P
Lactate dehydrogenase A	Lactate ↔ pyruvate
Fructose-bisphosphate aldolase A	Fructose-1,6P2 ↔ glyceraldehyde-3P + dihydroxyacetone-P
Glycerol 3-phosphate dehydrogenase 2	Dihydroxyacetone-P ↔ glycerol-3P
Enolase I	Glycerate-2P ↔ phosphoenolpyruvate
Triosephosphate isomerase I	Glyceraldehyde-3P ↔ dihydroxyacetone-P
Phosphoglycerate mutase	Glycerate-3P ↔ glycerate-2P

All eight enzymes lead to the overproduction of pyruvate and lactate, which are then secreted in the medium for adjacent cancer cells to take up and utilize as an energy source

lular lactate concentration goes too high, some of it can be secreted to prevent the pH from dropping too low [28]. Cancer cells adjacent to autophagic CAFs upregulate TP53-induced glycolysis and the apoptosis regulator (TIGAR). TIGAR is capable of protecting cancer cells against oxidative stress by inhibiting autophagy and apoptosis while simultaneously shifting cells towards oxidative phosphorylation (OXPHOS) and away from aerobic glycolysis [29]. Finally, several antioxidant enzymes, such as peroxiredoxin-1, have been observed to be upregulated in certain cancer cells [2]. It is likely that as more experiments are performed involving the TME and cancer cells, more evasion mechanisms will be elucidated.

4 Loss of Stromal Cav-1 Is an Indicator of Poor Prognosis in Breast Cancers

The importance of Cav-1 in transdifferentiating normal fibroblasts into myofibroblasts is well established. Recent experiments have shed light on the complex mechanisms by which cancer cells modulate their environment and manage to downregulate Cav-1 expression in fibroblasts. Cav-1 inhibits TGF-β type I receptor kinase. The lack of Cav-1 expression in the Cav-1(−/−) null skin fibroblasts can induce a myofibroblastic phenotype. One of the most widely known tumor-derived factors involved in the activation of CAFs is TGF-β1 [1, 30, 31]. Interestingly, in the absence of CAFs, TGF-β itself in cancer cells has no direct effect on cancer proliferation and survival [31]. It is believed that cancer-derived TGF-β acts in a paracrine manner and causes the downregulation of Cav-1α in CAFs. This event results in the overexpression of ROS by CAFs that can act both in an autocrine and a paracrine fashion, stimulating themselves and nearby fibroblasts to acquire a myofibroblastic phenotype. ROS inhibit prolyl hydroxylase (PHD) from targeting the transcription factor HIF-1α for degradation [32–34]. As a result, HIF-1α gets stabilized and translocated into the nucleus causing the overexpression of autophagy genes, BNIP3 (BCL2/adenovirus E1B 19 kDa protein-interacting protein 3) and BNIP3L (BCL2/adenovirus E1B 19 kDa protein-interacting protein 3-like), which compete with Beclin-1. Beclin-1 then acts as a mitophagy/autophagy factor causing the dysfunction of mitochondria and, thus, the increase of ROS, acting on a positive feedback loop [35, 36]. HIF-1α also upregulates CTSB (cathepsin B) and ATG16L1 (autophagy-related 16 like 1), which are markers for autophagy and mitophagy, respectively [29]. Additionally, TGF-β causes the upregulation of BNIP3, BNIP3L, and CTSB (cathepsin B), all of which can induce mitophagy/autophagy and therefore shift the cell away from OXPHOS and towards aerobic glycolysis [31]. BNIP3, BNIP3L, and CTSB increase lactate production, whereas ATG16L1 increases ketone production [37]. Lactate and ketone bodies can then be utilized by cancer cells to enhance tumor growth [37]. TGF-β, therefore, promotes tumorigenesis via CAF metabolism, and specifically TGF-β in fibroblasts

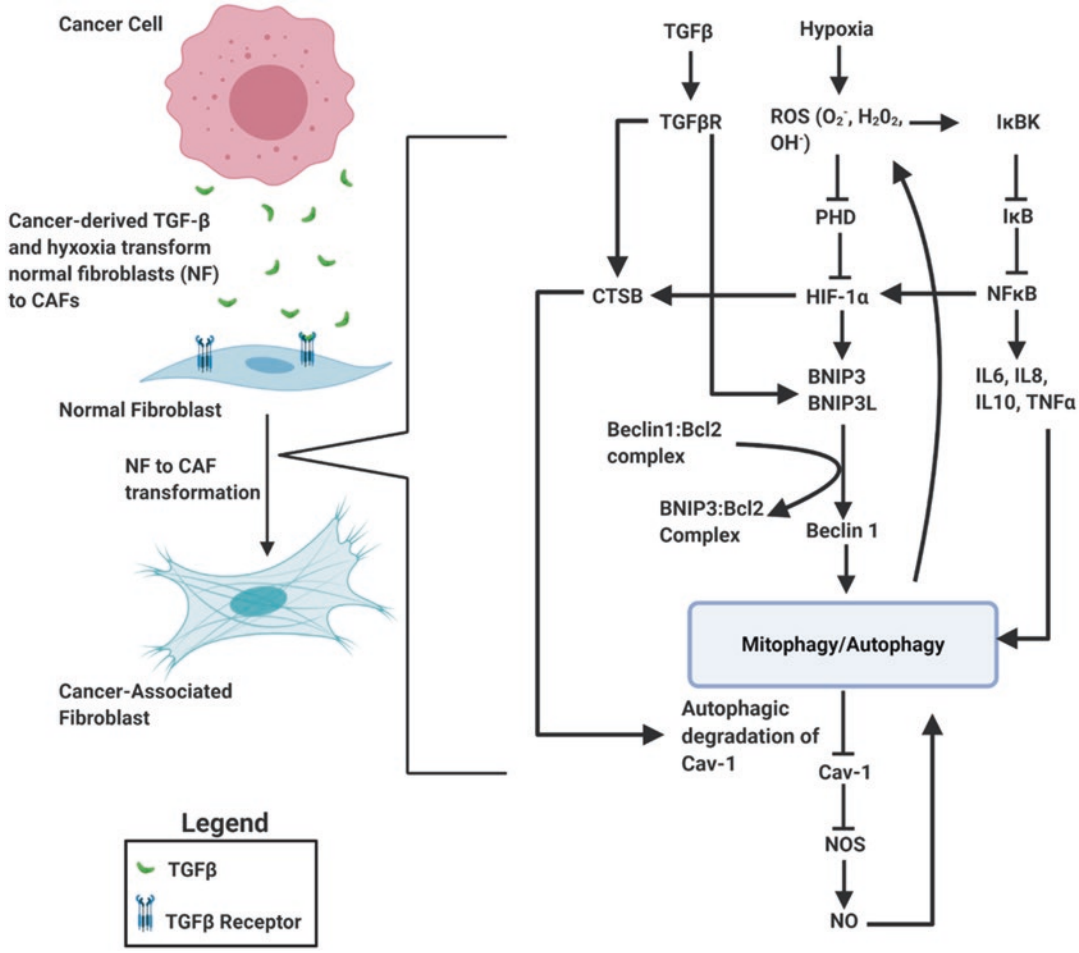

Fig. 1 Conversion of normal fibroblasts (NF) to cancer-associated fibroblasts (CAF) through cancer-induced hypoxia and cancer-derived TGFβ

leads to the upregulated mitochondrial activity of cancer cells and tumor growth [31] (Fig. 1).

The rapid proliferation of cancer, without a significant increase in vascularization, limits oxygen availability for normal fibroblasts, thus creating a hypoxic environment that forces the fibroblasts to undergo metabolic changes [1]. Hypoxia results in the stabilization of HIF-1α, which, as described previously, is a very important transcription factor for genes involved in autophagy, mitochondrial biogenesis, and general energy homeostasis [29]. Furthermore, under normoxia, NFκB (nuclear factor kappa-light-chain-enhancer of activated B cells), a key inducer of autophagy, is inhibited by the inhibitor of NFκB (IκB). IκB achieves this by sequestering the nuclear localization signal of NFκB, therefore rendering it inactive in the cytoplasm [38]. However, hypoxic conditions activate IκB kinase (IκBK), which targets IκB for degradation by phosphorylation and thus promotes the activation of NFκB [39]. Even though the exact mechanism by which NFκB is able to direct autophagy is unclear, it is believed that this transcription factor upregulates the expression of certain inflammatory cytokines, such as IL-6, IL-8, IL-10, and TNFα [40, 41]. These inflammatory mediators are able to induce autophagy independent from each other [40, 41]. Finally, NFκB also binds to the HIF-1α promoter and results in its upregulation [42] (Fig. 1). Hypoxia- and TGF-β-

induced autophagy cause the lysosomal degradation of Cav-1 as well as mitochondrial dysfunction and degradation, leading to a highly glycolytic state in CAFs. Cav-1α normally inhibits nitric oxide synthase and prevents the accumulation of nitric oxide (NO). In the absence of Cav-1α, NO accumulates and inhibits cytochrome c oxidase, causing mitochondrial uncoupling and thus rendering mitochondria susceptible to mitophagy [43]. This results in high amounts of lactate, pyruvate, ketone bodies, glutamine, and free fatty acids [2, 24, 44] that can be utilized by adjacent cancer cells.

The aforementioned oxidative stress and hypoxia derived from Cav-1 loss lead to mitochondrial dysfunction and degradation. Mitochondrial dysfunction causes the premature reaction of electrons with oxygen, leading to the generation of ROS, such as O^{2-}, H_2O_2, and OH· [2]. ROS induce oxidative stress, stabilize HIF-1α, and inhibit NFκB in a positive feedback manner. The fact that TGF was not able to stimulate a significant increase in angiogenesis and vascularization suggests that the growth stimulated by CAFs depends on the paracrine supply of high-energy molecules such as lactate, pyruvate, ketone bodies, amino acids, and fatty acids [45, 46].

5 miRNAs Play a Crucial Role in CAF Metabolic Reprogramming

MicroRNAs (miRNAs) are short noncoding RNAs that target mRNA and, therefore, can regulate gene transcription at the posttranscriptional level [47]. miRNAs have been shown to be upregulated in CAFs, as well as secreted by various tumors into the TME in microvesicles (MV) [47–51].

5.1 The Role of Endogenous miRNAs in the Metabolic Reprogramming of CAFs

Isocitrate dehydrogenase 3α (IDH3α), the enzyme responsible for the conversion of isocitrate to α-ketoglutarate, is downregulated in colon cancer CAFs and melanoma CAFs following TGFβ exposure [48]. This downregulation of IDH3α is attributed to the increased levels of miR-424 which leads to the accumulation of succinate and fumarate, which in turn inhibits the activation of PHD2 (the predominant enzyme that degrades HIF-1α by hydroxylation), thus leading to stabilization of HIF-1α [48, 52]. It is this HIF-1α stabilization and activity that leads to the upregulation of transporters and various glycolytic enzymes, such as glucose transporter 1 (GLUT1), hexokinase 2 (HK2), and 6-phosphofructokinase, muscle type (PKFM) [48], and causes an increase in glucose uptake and lactate production, as well as a decrease in oxygen consumption by these fibroblasts [48].

Additionally, HIF-1α in colon cancer CAFs and melanoma CAFs is able to inhibit OXPHOS by downregulating mitochondria complex I through the overexpression of a complex I inhibitor, known as NADH dehydrogenase 1 alpha subcomplex 4-like 2 (NDUFA4L2) [48]. It is noteworthy that the expression of NDUFA4L2 is TGFβ dose and time dependent [48].

In another set of experiments, miR-21 was shown to play a critical role in pancreatic cancer CAF development [49]. Metabolic coupling was evident between CAFs that had higher expression of miR-21 and pancreatic cancer cells. miR-21 upregulation increases glucose uptake and lactate production in CAFs, while at the same time upregulating SDH and FH in pancreatic cancers [49].

5.2 The Role of Exogenous miRNA in the Metabolic Reprogramming of CAFs

Yao et al. studied the interaction between pancreatic cancer cells and fibroblasts in 2015 [47]. They identified that miR-155 was packaged in MVs and secreted by pancreatic cancer cells [47]. Once in NFs, miR-155 exerted its transformative role by targeting the TP53INP1 gene and transforming NFs into CAFs [47, 50]. The transformative ability of miR-155 could be explained by the fact that TP53INP1 has pro-apoptotic properties and its downregulation by miR-155 leads to decreased mitophagy and accumulation of dysfunctional mitochondria that produce high levels of ROS [47, 51].

Table 2 Amino acids present in various CDEs

Amino acid	CDE from prostate CAF	CDE from pancreatic CAF
Alanine	Yes	Yes
Anserine		Yes
Arginine	Yes	Yes
Asparagine	Yes	
Citrulline	Yes	
Cysteine	Yes	Yes
Glutamic acid	Yes	Yes
Glutamine	Yes	Yes
Glycine	Yes	Yes
Histidine	Yes	Yes
Isoleucine		Yes
Leucine	Yes	Yes
Lysine	Yes	Yes
Methionine	Yes	Yes
Ornithine	Yes	Yes
Phenylalanine	Yes	Yes
Phosphoserine	Yes	
Proline	Yes	Yes
Serine	Yes	Yes
Threonine	Yes	Yes
Tryptophan		Yes
Valine	Yes	Yes

Table 3 The ten most abundant miRNAs present in CDE and their respective target genes

Exosomal miRNA	OXPHOS gene silenced
miR-302d-3p	UQCRFS1
miR-29b-3p	NDUFA10, ATP5G1, ATP6V1A, ATP5G3
miR-22-3p	ATP6V1A
miR-155-5p	ATP5G3
miR-25-3p	NDUFS4
miR-29a-3p	ATP5G1, ATP6V1A, ATP5G3
miR-23a-3p	UQCRFS1, NDUFS4, ATP6V0E2, NDUFA2
miR-21-5p	ATP5L, ATP5G2
miR-16-5p	ATP5G3
miR-222-3p	ATP6V1A

6 CAF-Derived Exosomes (CDEs) Can Reprogram the Metabolic Pathway of Cancer Cells

Much research has been focused on exosomes secreted by cancer cells, while little is known about exosomes secreted by CAFs. Zhao et al., with the use of isotopologue tracing [53], showed that CAF-derived exosomes (CDEs) are taken up by cancer cells in a *KRAS*-independent mechanism and are, indeed, capable of reprogramming the metabolic activity of pancreatic and prostate cancer cells [54]. They demonstrated how CDE can sustain the rapidly dividing cancer cells under hypoxic conditions or when the normal oxidative mitochondrial function has been disabled. Additionally, the presence of CDEs can rescue prostate and pancreatic cancer cells from starvation by providing de novo-synthesized metabolites, such as amino acids (Table 2). This suggests that there is constant communication between the cancer cells and the adjacent fibroblasts, where both constantly coevolve [54].

6.1 CDEs Contain miRNAs that Downregulate Oxidative Phosphorylation of Cancer Cells

CAF-derived exosomes (CDEs) contain amino acids, fatty acids, pyruvate, lactate, miRNA, and many other compounds. miRNAs are essential in regulating gene expression [54]. Zhao et al. showed that miRNAs present in CDEs are capable of downregulating all 109 OXPHOS-related genes in cancer cells. As shown in Table 3, the ten most abundant miRNAs present in these CDEs target one or more OXPHOS genes, leading to decreased OXPHOS. Therefore, cancer cells must rely on alternative metabolic pathways to maintain their rapid proliferation [54].

6.2 Effect of CDEs on Glycolysis and TCA of Cancer Cells

With the use of GC-MS and $^{13}C_6$-glucose, Zhao et al. showed that glucose from CDEs was the main glycolytic substrate for cancer cells. This was evident due to the increase in labeled glycolytic metabolites, lactate and pyruvate, in prostate cancer cells cultured with CDEs, and the reduced amount of non-labeled pyruvate and lactate (Fig. 2). Additionally, they further

showed that the labeled metabolites involved in the TCA cycle (citrate, α-ketoglutarate, fumarate, and malate) are found in significantly lower concentrations when cultured with CDEs. Therefore, the increase of labeled glycolytic metabolites and the decrease of labeled TCA metabolites suggest that glucose provided by CDEs is primarily used in glycolysis and not in mitochondrial oxidative phosphorylation [54].

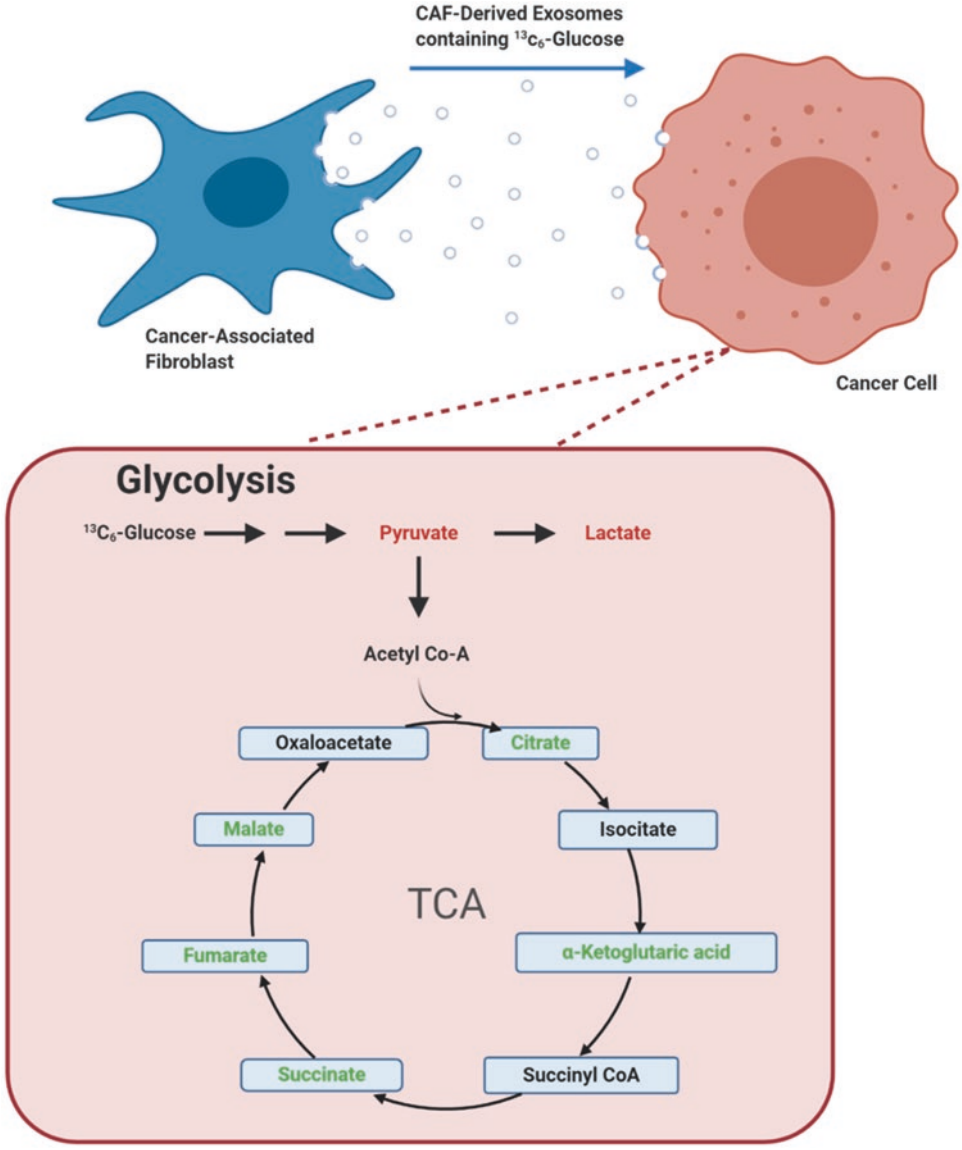

Fig. 2 CDE-derived glucose is mainly used in cancer cell glycolysis and, to a lesser extent, the TCA cycle. Metabolites in red represent the metabolites found in cancer cells present in high concentrations resulting from CDE-derived glucose. Metabolites in green represent the metabolites found in cancer cells present in low concentrations from CDE-derived glucose

6.3 Glutamine from CDEs Undergoes Mainly Reductive Metabolism that Also Results in Aberrant Lipogenesis in Adjacent Cancer Cells

Glutamine is another major carbon source for the TCA cycle and a nitrogen source for protein synthesis [55–58]. Zhao et al. identified the contribution of CDE-derived glutamine to the TCA cycle in the cancer cells using U-$^{13}C_5$-glutamine isotopologue tracing [54]. Under both normoxic and hypoxic conditions, glutamine can enter the oxidative metabolic pathway and produce oxaloacetate, which then combines with acetyl-CoA to form citrate, a fatty acid precursor. Additionally, under hypoxic conditions, glutamine enters the reductive metabolic pathway generating α-ketoglutarate and then citrate [59]. As shown in Fig. 3, citrate is eventually converted to fumarate and then malate. The presence of M + 5 citrate, M + 3 fumarate, and M + 3 malate in high concentrations suggests that cancer cells mainly rely on the reductive glutamine metabolism when the normal mitochondrial function of the cell is disrupted. Additional evidence to support this is the decreased M + 4/M + 5 citrate ratio. M + 4 citrate is derived from the oxidative pathway of glutamine, whereas M + 5 citrate is from the reductive pathway, and therefore this reduced M + 4/M + 5 citrate ratio confirms the predominance of the glutamine reductive pathway [54]. Furthermore, a major component and requirement for cell proliferation is lipogenesis, the generation of fatty acids for cell membranes [60]. Zhao et al. also showed that exposure to CDEs resulted in increased acetate contribution and simultaneously decreased pyruvate contribution to lipogenesis. This event suggests that the main source of carbon for acetyl-CoA upon exposure to CDEs is the glutamine reductive carboxylation pathway and not the oxidative glucose pathway. Finally, metabolic analysis of CDEs revealed significant amounts of stearate and palmitate that can be directly utilized by the cancer cells for lipid synthesis [54]. It is worth mentioning that fatty acid synthase (FASN) expression has been found to be elevated in numerous types of cancer [61]. Even though there is still no direct link between CAFs and overexpression of FASN, this could be the result of the coevolution of stroma and cancer. However, more research is required before conclusions can be drawn.

7 CAF-Derived Lactate Is more Than Just a Metabolite

Sirtuins (SIRTs) are deacetylases that are activated when NADH/NAD+ ratios are unbalanced and act as sensors of nutrient deprivation [62–64]. SIRT1, specifically, targets and activates peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), a transcription factor that promotes mitochondrial respiration and OXPHOS [65–68]. CAF-derived lactate is converted to pyruvate by the upregulated enzyme lactate dehydrogenase B (LDHB), leading to the accumulation of NADH. High levels of NADH result in the increased expression of SIRT1. Currently, lactate is the only metabolite capable of inducing SIRT1 activation and PGC-1α deacetylation and therefore proves the importance of lactate as more than just a metabolite. NADH is then oxidized back to NAD+, and the unbalanced high levels of NAD+ cause the activation of SIRT1, thus help maintain the increased mitochondrial activity and OXPHOS in cancer cells [69].

As mentioned earlier, increased mitochondrial activity and oxygen consumption have been recorded in many tumors when co-cultured with CAFs. Prostate cancers are an example [69]. CAF-derived lactate enters the TCA cycle, leading to the accumulation of citrate, succinate, fumarate, and malate, but not to a significant increase in α-KG [69]. Additionally, in prostate cancer cells, mitochondrial complexes II–III are downregulated, and complex I is upregulated, leading to the accumulation of mitochondrial reactive oxygen species (mtROS) [69]. The increased complex II dysfunction leads to the accumulation of succinate [69].

mtROS oxidize and activate Scr, a crucial and mandatory step for CAF-derived lactate addiction of prostate cancer cells [69]. Scr is able to sustain the activation of SIRT1/PGC-1α.

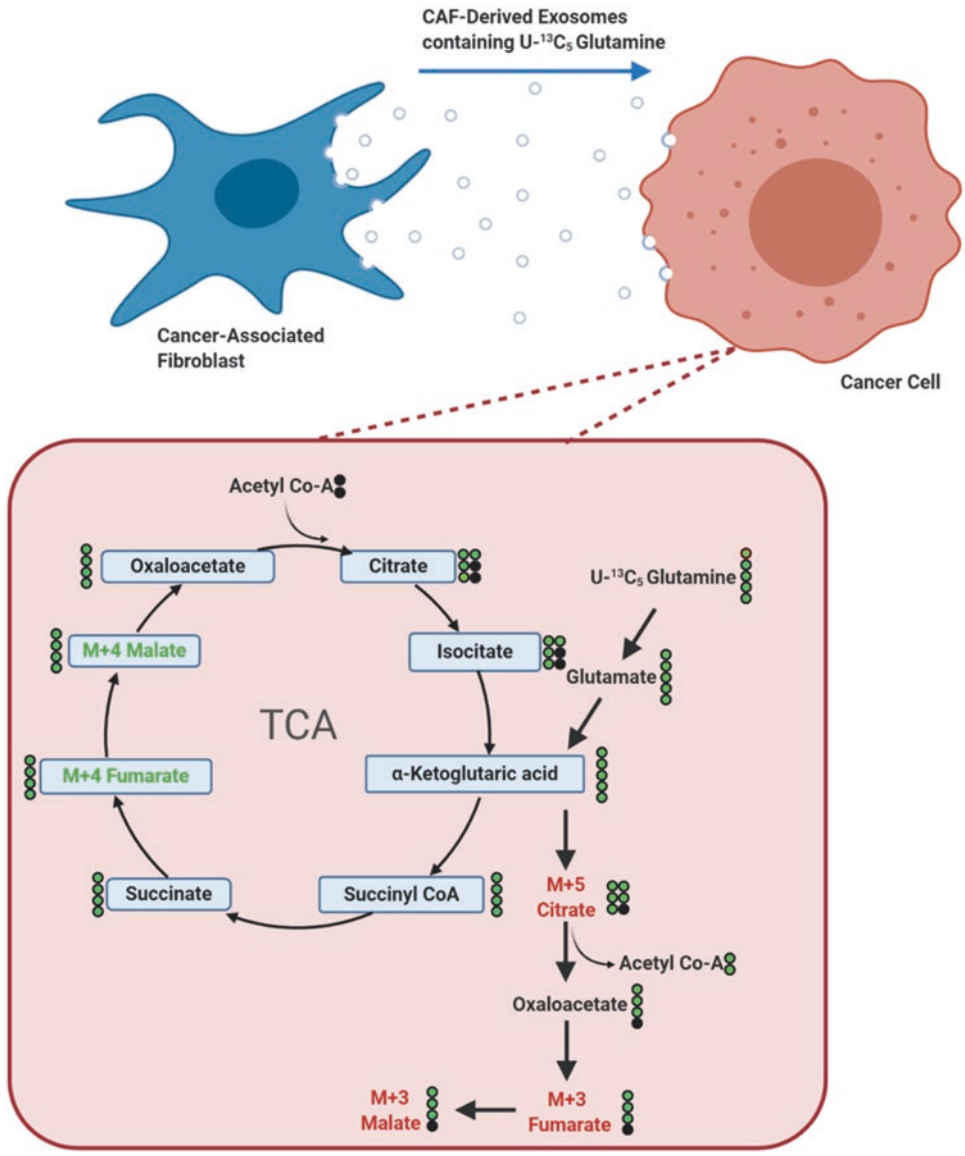

Fig. 3 Oxidative and reductive glutaminolysis. Green circles represent labeled carbons (^{13}C), and black circles represent non-labeled carbons (^{12}C). Glutamine derived from CDEs is fully labeled, and its subsequent pathways (oxidative or reductive) in cancer cells are analyzed. Metabolites in red represent the metabolites of the reductive metabolic pathway of glutamine found. Metabolites in green represent the metabolites of the oxidative metabolic pathway of glutamine found

In the absence of Scr activity, prostate cancer cells are unable to utilize CAF-derived lactate in this manner and show no SIRT1/PGC-1α activity [69].

Even though the increase in both mtROS and PGC-1α might seem controversial since PGC-1α is a regulator of the antioxidant response, it is believed that there is a fine balance between the two that helps aid prostate cancer survival and promotes OXPHOS [69]. PGC-1α could be acting in a negative feedback loop, ensuring that mtROS remain under control [69]. Taken together, CAF-derived lactate is a crucial metabolite in prostate cancer metabolic transformation

towards OXPHOS through mtROS production, Src activation, and SIRT1/PGC-1α activity.

8 CAFs "Surrender" Their Functional Mitochondria to Prostate Cancer Cells

In vitro experiments showed that CAFs are able to surrender their functional mitochondria to prostate cancer cells [69]. This effect is more prominent in higher grade prostate tumors [69]. Interestingly, after exposure to CAF-conditioned media, significantly more prostate tumor cells were able to receive CAF-derived mitochondria. This suggests that CAFs act in a paracrine manner to prime prostate cancer cells and prepare them to receive functional mitochondria [69]. Additionally, confocal microscopy and imaging techniques were able to show the transfer of mitochondria from CAFs to cancer cells through nanotubule formation. NFs were unable to donate their mitochondria, suggesting that this is a property limited to CAFs and not to all fibroblasts [69].

Prostate cancer cells that received CAF-derived mitochondria express higher levels of MCT1, the transporter that transports lactate into the cell. Those prostate cancer cells also upregulate SIRT-1 and PGC-1α. Interestingly, inhibition of MCT1 prevents the receipt of CAF-derived mitochondria. The exact mechanism and relation of MCT1 with the mitochondrial transfer are still unknown, but its importance in enhancing OXPHOS metabolism in mitochondrial recipient prostate cancer cells is evident [69]. An increased number of mitochondria and OXPHOS can aid higher grade tumors to meet the high energy demands [69].

9 CAFs Augment Cancer Addiction to Glutamine and Its Metabolically Relevant Consequences

As mentioned earlier, there exists constant coevolution between cancer cells and the TME. Cancer cells stop directing glucose into the TCA cycle and, instead, use glucose for the production of nucleotides [70]. Consequently, cancer cells start relying on other carbon sources for oxidative phosphorylation, particularly on glutamine derived from CAFs [54]. However, during glutaminolysis, specifically during the conversion of glutamine to glutamate and then to α-ketoglutarate (which enters the TCA cycle), ammonia is released as a by-product [57, 71, 72]. Ammonia is a diffusible compound and an inducer of autophagy. This has detrimental effects on the surrounding stroma, as it causes autophagy in the adjacent CAFs. CAFs, subsequently, undergo autophagy and further release glutamine to be metabolized by the cancer cells. Therefore, a positive feedback loop exists between cancer's addiction to glutamine and CAF's conversion/autophagy [71, 72].

10 Alanine Secreted by Pancreatic Stellate Cells Supports Pancreatic Cancer Metabolism

In one study, Sousa et al. discovered that myofibroblast-like pancreatic stellate cells (PSCs) secreted alanine and were able to support pancreatic cancer metabolism [70]. Among the 200 metabolites analyzed, only alanine and aspartate met the following criteria: (1) increased amounts of metabolite in PSC medium, (2) decreased amounts of metabolite in PSC medium when exposed to pancreatic cancer (PDAC) cells, and (3) increased amounts of metabolite in PDAC after exposure to PSC medium. Furthermore, kinetic studies showed that alanine is secreted even more rapidly than lactate. In fact, PSC-derived alanine does not contribute to the production of glycolytic intermediates or alter the NAD+/NADH ratio, but rather, it is transaminated to pyruvate, providing additional substrates for the TCA cycle. This intermediate contribution to the TCA cycle subsequently increases oxygen consumption [70]. Alanine-derived pyruvate enters the TCA cycle in the mitochondria and contributes predominantly to the generation of citrate (23–46% among different PDAC cell lines) and

isocitrate. To a lesser extent, it also contributes to the generation of malate, fumarate, aspartate, and glutamate. Alanine, therefore, fuels mitochondrial metabolism without affecting glycolysis. Alanine-derived citrate is then transported from the mitochondria to the cytosol for lipogenesis. Metabolite tracing showed that alanine significantly contributed to the generation of palmitate and stearate, more than 20% and 10% of the total concentrations, respectively [70]. In the presence of alanine, glucose enters the serine biosynthetic pathway in PDAC cells and produces serine and glycine. Serine and glycine can then be used in the biosynthesis of nucleic acids. Under nutrient-deprived conditions, the entry of glucose into the serine biosynthetic pathway is more evident. This suggests that in cases of glucose-deprived conditions, alanine can take over aerobic respiration by providing TCA cycle intermediate metabolites, and subsequently, glucose can then enter different metabolic pathways, such as the serine biosynthetic pathway [70].

The induction of alanine secretion by PSCs for PDAC cells to take up is a two-way intratumoral cross talk. PDAC cells initially stimulate PSCs to undergo autophagy and thus lead to the release of alanine. PSC-derived alanine is then taken up by PDAC cells to contribute to metabolic pathways. In nutrient-rich conditions, the PSC autophagic alanine secretion has a minimum effect on PDAC proliferation. However, in nutrient-deprived conditions, PSC autophagic alanine secretion can significantly rescue and promote the growth of PDAC cells. This effect mainly occurs during the early stages of cancer development. Interestingly, autophagy does not influence the proliferation rate of PSCs themselves [70].

11 CAFs Act as Lipid Synthesis Factories for Colorectal Cancer Cells

New research performed by Zhao et al. in 2020 has shown that CAFs overexpress FASN and undergo lipidomic reprogramming to help colorectal cancer cells meet their high energy demands by providing them with newly synthesized lipids [73]. Nineteen lipids were specifically identified that were produced and secreted by CAFs and taken up by colorectal cancer cells [73]. However, more research is needed to identify the exact mechanisms that stimulate this lipidomic reprogramming in CAFs and how the de novo lipids are utilized by cancer cells [74].

12 Reciprocal Communication Is Essential for Cancer Progression

The importance of *KRAS* in supporting heterocellular communication was demonstrated by Tape et al. [25]. When PDAC cells were exposed to homocellular conditions, mitochondrial function decreased, and superoxide concentrations increased. However, when PDAC cells were exposed to heterocellular conditions (i.e., co-cultured together with CAFs), mitochondrial function was restored, and superoxide concentrations were well regulated. These results suggest that heterocellular and reciprocal communication between CAFs and cancer cells is essential for the progression of cancer. In this experiment, PDAC cells with *KRAS* mutation initially stimulated the surrounding CAFs to undergo metabolic and cellular changes. Reciprocal stimulation between CAFs and PDAC cells prevents cancer cell mitochondrial dysfunction and superoxide production. The exact signals involved in this dialogue between CAFs and PDAC cells are still unclear, and further research is required to unravel this mechanism [25].

13 Conclusion

As cancer research progresses, the significance of the TME in cancer progression is better elucidated [75]. Tumor cell-derived TGF-β causes the lysosomal targeting of fibroblastic Cav-1, inducing a myofibroblastic phenotype (activated form). Additionally, the increased oxygen consumption of cancer cells with no significant increase in vascularization induces hypoxia and oxidative stress, causing the stabilization of HIF-1α and inhibition of IκB in CAFs. Stabilized HIF-1α induces

autophagy and mitophagy. Subsequently, CAFs rely on glycolysis for energy, producing a high amount of lactate, ketone bodies, glutamine, and fatty acids, which are then secreted and taken up by the surrounding cancer cells.

In the case of PDAC, it is evident that the secretion of alanine by PSCs (myofibroblast-like pancreatic stellate cells) is sufficient to rescue cancer cells in low-nutrient environments. It was noted that alanine, not glucose, is used in the TCA cycle. This allows glucose to enter the serine biosynthetic pathway to generate nucleic acids, further contributing to the rapid cancer cell proliferation.

Furthermore, miRNAs play a significant role in metabolically coupling CAFs and various cancers, such as pancreatic cancer. miRNAs can lead to increased glucose uptake and lactate production in CAFs, while at the same time SDH and FH are upregulated in pancreatic cancers. CAFs have also been demonstrated to "surrender" their functional mitochondria to prostate cancer cells to help cancers meet their high energy demands. In other cases, such as with colorectal cancer, CAFs act as lipid synthesis factories.

CAFs' contribution to cancer progression does not end here. Exosomes derived from CAFs (CDEs) contain a variety of miRNAs responsible for downregulating genes involved in OXPHOS and therefore contribute to the reprogramming of the metabolic activity of cancer cells. CDEs also contain de novo metabolites that enable the rapidly dividing cancer cells to survive in low-nutrient conditions. For example, CDE-derived glutamine undergoes reductive metabolism and generates acetyl-CoA for lipogenesis. However, during glutamine metabolism, ammonia, a diffusible autophagy factor, is produced as a by-product. CAFs that are stimulated by ammonia undergo autophagy and, in turn, further release more glutamine to be metabolized by cancer cells in a positive feedback loop. It is clear that cancer should not be regarded as an individual entity anymore, but rather it should be viewed within the context of its microenvironment. The understanding of the extent of the stromal impact on cancer metabolism and progression can provide new targets for cancer therapy [76].

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