Mitophagy and Reverse Warburg Effect: Metabolic Compartmentalization of Tumor Microenvironment

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Abstract

'The Warburg effect' is one of the aberrant glucose metabolism pathways in cancer cells that generate malignant phenotypes and promotes cancer progression. However, in the year 2009, a novel model called 'two-compartment metabolic coupling' model or 'the reverse Warburg effect' was proposed where the tumor stromal plays a crucial role in the process of tumor progression. Based on this new model, the present review summarizes the autophagic stroma model of cancer and multiple compartment model of tumor metabolism. Cancer-associated fibroblast cells in tumor microenvironment undergo aerobic glycolysis (the reverse Warburg effect) just like the cancer cells. Such a phenomenon is possible only due to the forced activation of glycolysis by decreasing the mitochondrial mass and/or generating dysfunctional mitochondria. The tumor stroma is often found with autophagic and mitophagic activities as evidenced by the higher expression of autophagic and mitophagic signature molecules. Moreover, caveolin-1 and hypoxia-inducible factor-1α play a fundamental role in governing the mitophagy-mediated occurrence of 'reverse Warburg effect'. To the surprise, cancer stem cell also follows the same strategy to exploit the tumor stroma in order to derive high energy fuels for its survival and proliferation. Such parasitic energy-coupling between the cancer cell and cancer-associated fibroblasts makes the fibroblasts a metabolic slave. The metabolic coupling is the result of the paracrine regulation where oxidative stress generated in adjacent fibroblasts by the reactive oxygen species (ROS) produced by cancer cells along with the up-regulation of the oncometabolite transport process through various transporters. This review also discusses the paradigm shift from 'the Warburg effect' to 'the reverse Warburg effect'. It also describes the pivotal role of mitophagy in triggering the 'the reverse Warburg effect'.

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6.1 Introduction

Recently, tumor microenvironment (TME) has gained greater attention due to its roles as an essential contributing factor in the progression of cancer as well as its association with poor clinical outcomes. Moreover, tumors are very often regarded as organs owing to their distinct vasculature wherein cancer cells are protected by a protumor microenvironment (Egeblad et al. 2010). It is now obvious that tumor is not a pure homogeneous population in vivo. Rather, the cells in tumor are set in 'cancer cell nests', which together constitute tumor microenvironment. TME comprises (1) extracellular matrix (ECM), (2) cancer cells, (3) cancer stem cells (CSCs), (4) infiltrating immune cells [B lymphocytes and T lymphocytes, eosinophil, neurophill, basophil, natural killer (NK) cells, mast cells, antigen presenting cells (APC) (dendritic cells and macrophages) and tumor-associated macrophages (TAMs)], (5) Stromal cells [fibroblast cells, myofibroblasts and cancer-associated fibroblasts (CAFs)], and (6) angiogenic endothelial cells [Tumour associated endothelial (TAE) cells] along with their precursors (pericytes) (Friedl and Alexander 2011; Hanahan and Coussens 2012). The TME is a discrete and dynamic domain that guarantees well-defined functional attributes leading to the formation of a suitable habitat that protects cancer cells from various genetic and epigenetic insults and gives a favourable environment for growth and development. Many documents report the occurrence of a desmoplastic 'reactive stroma' encompassing CAFs and myofibroblast-like cells that provides a protumor microenvironment (Zhang et al. 2013). In this regard, it is believed that stromal fibroblasts manipulate the onco-metabolic processes and vice versa (Avagliano et al. 2018; Zhang et al. 2013). Interestingly, reports claim that fibroblasts cells when co-cultured with cancer cells lose their mitochondria. On the contrary, the cancer cells showed an increased mitochondrial mass. Such behavioural aspects of fibroblasts and cancer cells depict the host–parasite relationship where cancer cells act as 'parasites' and stromal fibroblast cells as 'host' (Ko et al. 2011; Martinez-Outschoorn et al. 2011b; Zhang et al. 2013). Similar to the previous reports on infectious 'intracellular' parasites which employs oxidative stress and autophagy to yield host-derived recycled nutrients, the cancer cells also behave as 'extracellular' parasites. Cancer cells exert oxidative stress which acts as a 'weapon' to induce autophagic elimination of mitochondria, by mitophagy to obtain nutrients from neighbouring stromal cells and are compelled to perform aerobic glycolysis to generate energy-rich metabolites (e.g. lactate and ketones) to 'feed' nearby cancer cells (Ko et al. 2011; Whitaker-Menezes et al. 2011a). This paradigm is referred to as 'The Autophagic Tumor Stroma Model of Cancer Metabolism' (Lisanti et al. 2010; Pavlides et al. 2010; Whitaker-Menezes et al. 2011a). Such a strategy employed by cancer cells

simply via inducing oxidative stress wherever they go allow them to seed anywhere and offers decreased dependency on blood vessels for food supply during metastasis. This model advocates the quintessential role of autophagy and mitophagy in the alterations of stromal catabolism to promote anabolic growth of cancer cells in order to promote survival advantage during cancer progression (Pavlides et al. 2012). Hence, antioxidants and autophagy inhibitors that have potential in uncoupling such parasitic metabolic relationships inside the tumor microenvironment would provide novel insights into cancer therapeutics.

6.2 Cancer Metabolism: Pasteur Effect, Inverted Pasteur Effect, Warburg Effect and Reverse Warburg Effect

In the 18th century, Antoine Lavoisier reported that to release energy, living organisms slowly burn the metabolic fuels by consuming oxygen. Later on, Louis Pasteur postulated the *'Pasteur effect'*, which proposes that 'fermentation is an alternate form of life and that fermentation is suppressed by respiration'. Six decades later, Warburg proposed that augmented glucose fermentation and diminished respiration is the chief ground of carcinogenesis. It was considered as a counter to the '*Pasteur effect*' and popularly known as '*aerobic glycolysis*'. However, another scientist cotemporary to Warburg named Crabtree demonstrated that aerobic glycolysis is used as an energy source during pathological overgrowths. Moreover, glucose uptake and glycolytic activity were shown to have a negative effect on oxygen consumption collectively known as '*inverted Pasteur effect*' or '*Crabtree effect*' (Vadlakonda et al. 2013). Intriguingly, it is the metabolic responses of tumor cells which permit them to thrive and establish in a particular microenvironment. Such metabolic adaptation noted in cancer cells controls the therapeutic responses. Here, tumor progression and metastasis are dependent on the metabolic adaptation of both cancer and non-cancerous cells present in the vicinity of tissue or organ. Moreover, the metabolism involves both the intracellular network that distributes and offers organic compounds, and the extracellular organic and signalling molecules that facilitate intercellular signalling, which in turn regulates the metabolic functioning of a cell. It is well known that the metabolic interactions among the tumor cells and the stromal cells provide survival advantages where the stromal cells facilitates metabolic substrates supplementation in the form of glutamine, lactate, and fatty acids.

6.2.1 Warburg Effect

Initially, the German chemist and physician Otto Warburg and colleagues in the 1920s performed experiments on the lactate production and oxygen consumption in tissues derived from liver carcinoma of rat (Warburg 1925). To their surprise, they found strikingly different glucose metabolism between normal and cancer cells. Moreover, cancer cells were found to be more reliant on glycolysis despite oxygen availability. This led to the discovery of 'Warburg effect' which referes to the

phenomenon of preferred aerobic glycolysis and enhanced lactate production despite the availability of sufficient oxygen in the vicinity. This hypothesis of Warburg proposes that usually cancer cells produce energy by non-oxidative glucose break down, i.e., 'Glycolysis'; whereas normal cells produce ATP through oxidative phosphorylation (OXPHOS) (Warburg 1956). Warburg effect is documented in many cancers including cancer of lungs, colorectal cancer, glioblastoma and breast. DeBerardinis et al. have experimentally proven the occurrence of Warburg effect by studying the cancer cells incubated with 10 mM C-13-labelled glucose under oxygenated conditions (DeBerardinis et al. 2007). Elevated levels of glycolytic metabolites were observed during the metabolomic analysis when 4 mM glucose was perfused to cancer cells prior to experiments suggesting the occurrence Warburg phenomenon (Fantin et al. 2006). Only 2 ATP per molecule of glucose is produced through glycolysis under the anaerobic condition, which is much lower than OXPHOS (i.e. 30 or 32 ATP per glucose molecule). This indicates that in comparison with aerobic glycolysis near about 15 times more glucose is needed to be anaerobically catabolized to generate the same amount of energy. As a result, tumor cells require more glucose, and to make that happen, there is a ten times faster uptake of glucose in tumor cells than the normal cells. It has been suggested that a lower yield but a higher rate of ATP production provides selective advantage to cells competing for limited and shared energy resources. Moreover, cancer cells compete with stromal cells and other cells in the tumor microenvironment due to limited availability of glucose (Chang et al. 2015; Pfeiffer et al. 2001; Slavov et al. 2014). Moreover, cancer cells prefer anaerobic glycolysis for production of ATP because of limited O_2 exposure and hypoxic conditions (Bartrons and Caro 2007). It has been found that high glucose levels in the culture media considerably reduces mitochondrial respiration and vice versa (Gohil et al. 2010; Marroquin et al. 2007). Under high (25 mM) and low (1 mM) glucose conditions, the cancer cells were cultured to investigate oxygen consumption rates (OCRs; mitochondrial respiration) and extracellular acidification rates (ECAR; glycolysis). It was noticed that upon culture under high glucose conditions, cancer cells showed either high OCR-low ECAR or low OCR-high ECAR, or high/moderate OCR-high/moderate ECAR. However, under low glucose conditions, the cancer cells showed high–moderate OCR with very little ECAR as other substrates are used for cellular ATP production (Potter et al. 2016). Moreover, Warburg effect is not consistent as seen in a study with rat hepatoma carried out by Weinhouse. According to this study, the slow-growing cells were more oxidative, whereas the more proliferative cells were more glycolytic. There occurs a dynamic interplay between oxidative and glycolytic states called as metabolic flexibility or metabolic plasticity (Jose et al. 2011; Obre and Rossignol 2015). Such metabolic flexibility is dependent on the environmental conditions and cancer-associated mutations (Astuti et al. 2001; Baysal et al. 2000; Dang et al. 2009; Yan et al. 2009). This kind of dynamic interplay of cancer cells is accompanied with mitochondrial dysfunction. Moreover, studies showed that an increased glycolytic rate is the consequence of decreased mitochondrial mass in cancer cells (Gogvadze et al. 2010).

Warburg effect has been explained in many cancer types and their role in cancer is proposed to be associated with transcriptional and post-translational related

metabolic changes. One of the transcription factors, hypoxia-inducible factor 1 (HIF-1) up-regulates expression of glycolytic enzymes, glucose transporters, and pyruvate dehydrogenase kinases (PDKs). Up-regulation of PDKs phosphorlyates and deactivates mitochondrial pyruvate dehydrogenase (PDH) complex and tricarboxylic acid (TCA) cycle (Semenza 2010). Many transcription regulators like alpha estrogen-related receptor (ERR) and MYC are associated with Warburg effect in the same manner (Yeung et al. 2008). Increased expression of MYC in tumors is proposed to be linked with an enhanced glycolytic rate and pathophysiology of metabolic modifications. MYC overexpression leads to high uptake of fluorodeoxyglucose (FDG) in human breast cancer (Palaskas et al. 2011). Again, MYC enhances the Warburg effect by elevating glucose flux and preventing the entry of pyruvate into the TCA cycle. Moreover, MYC overexpression is reported to enhance the activity of the glycolytic enzyme, transmembrane transport of glucose, glutamine transporters and glutaminase-1 activity (Dang et al. 2008; Gao et al. 2009; Nilsson et al. 2012; Osthus et al. 2000; Shim et al. 1997). The orphan nuclear receptor, ERR regulates oxidative metabolism, and mitochondrial biogenesis along with augmented glucose metabolism (Villena and Kralli 2008). Similarly, tumor suppressor protein p53 can lower the glycolysis rate by enhancing the enzymatic activity of fructose-2,6-bisphosphatase and thereby, increase the oxidative phosphorylation process. Warburg effect is also associated with a diminished level of expression of p53 in cancer cells linked with increased glycolysis (Bensaad et al. 2006; Maddocks and Vousden 2011). p53 is also shown to promotes OXPHOS by elevating cytochrome c oxidase and loss of expression of p53 in cancer cells therefore can induce the Warburg effect (Matoba et al. 2006). Moreover, the Warburg effect is also investigated for its association with post-translational regulation in cancer metabolism. Oncogenic phosphorylation events on metabolic enzymes promote aerobic glycolysis. It has been found that hexokinase (HK) and phosphofructokinase-2 (PFK-2) phosphorylation by AKT; downstream of PI3K activation facilitates glucose transporter (GLUT) expression and its localisation to the plasma membrane (Robey and Hay 2009). Studies on various cancer models have depicted the relationship of glycolysis and post-transcriptional modification of the M2 isoform of pyruvate kinase (PKM2). Post-translational modifications of PKM2 like the K305 acetylation decreases its enzymatic activity and modifies the glycolytic pathway. Moreover, it leads to increased degradation of such enzymes by activating chaperone-mediated autophagy. The expression of Y105F mutant of PKM2 in tumor cells was shown to have reduced lactate production and increased oxygen consumption which was consequently found to induce tumor xenograft development (Hitosugi et al. 2009). Again, the induction of PI3K/AKT pathway led to an increase in the phosphorylation of PFK-2, and HK and enhanced glucose influx; subsequently up-regulating the glycolytic pathway (Høyer-Hansen and Jäättelä 2007; Zheng et al. 2011). As mentioned earlier, high demand for glucose is an important feature of cancer and in tumors, there is enhanced relative uptake of FDG, or fluorodeoxyglucose F 18 (18F-FDG). However, it has been found that 18F-FDG uptake is considerably high in hypoxic cancer cells than normoxic ones. Moreover, 18F-FDG uptake in the normoxic cancer cells is typically low and is

similar to cells of stromal or necrotic regions leading to the question whether the Warburg effect actually applies to normoxic cancer cells or not. It has also been reported that there is an augmented increase in 18F-FDG uptake in hypoxic cancer cells than the normoxic ones in both in vivo and in vitro culture studies. Hence, cancer cells are supposed to have increased demand for glucose in the absence of oxygen which is logically explained by the Pasteur effect (Zhang et al. 2015).

6.2.2 The Reverse Warburg Effect

Previously, it was believed that the Warburg effect is a phenomenon performed only by cancer cells. However, human skin keloid fibroblasts were also shown to produce energy in the form of ATP mostly via glycolysis. Similarly, hypoxic microenvironments in tumors and keloids led to the activation of the same phenomenon (Vincent et al. 2008). Firstly, Pavlides et al. (2009) described that hydrogen peroxide (H_2O_2) released by cancer cells could induce oxidative stress in CAFs resulting in the loss of mitochondrial function leading to a metabolic switch from OXPHOS to glycolysis. Subsequent to the glycolytic switch, the lactate production by CAF accelerates (Zong et al. 2016). Lactates are transported to extracellular space via monocarboxylate transporter 4 (MCT4) and taken up by the cancer cells by MCT1 for its use in oxidative metabolism (Martinez-Outschoorn et al. 2011a, 2013). Furthermore, many co-culture systems involving fibroblast cells and cancer cells were experimented in this regard and it was observed that epithelial cancer cells are potentially capable of inducing the Warburg effect in stromal fibroblasts (Martinez-Outschoorn et al. 2011a). This phenomenon is popularly regarded as 'reverse Warburg effect' (RWE) (Fig. 6.1) (Jiang et al. 2019; Pavlides et al. 2009). Basically, the hypoxic and nutritionally challenged tumor microenvironment exploits CAFs as 'metabolic slaves' (Roy and Bera 2016). Interestingly, it is to be noted down that in the current situation stromal fibroblasts cells and not the cancer cells are undertaking the Warburg effect. The reverse Warburg effect was proposed to explain metabolic flexibility as mentioned earlier. To understand it better, it can be explained in two steps. In the first step, the cancer cells educate CAFs to boost aerobic glycolysis which leads to the enhanced production of energy-rich fuels (e.g. pyruvate, ketone bodies, fatty acids and lactate). Furthermore, in the second step, these energy-rich fuels produced by CAFs are utilized in mitochondrial OXPHOS by the cancer cells. Particularly, the lactates are converted to pyruvate by lactate dehydrogenase-B (LDH-B) enzymes (Martinez-Outschoorn et al. 2010b). It is also found that in normoxic microenvironment, the oxidative tumor performs OXPHOS to leave behind glucose for its utilization by glycolytic cancer cells in the hypoxic microenvironment (Bonuccelli et al. 2010; Feron 2009; Porporato et al. 2011; Sandulache et al. 2011; Sonveaux et al. 2008; Wilde et al. 2017). In other words enhanced lactate production is utilized in mitochondrial OXPHOS in cancer cells for energy production. This is also accompanied by diminished expression of caveolin-1 (Cav-1) in stromal cells. According to report, the Cav-1 is an important structural protein that plays an essential role in endocytosis, vesicular transport, and other

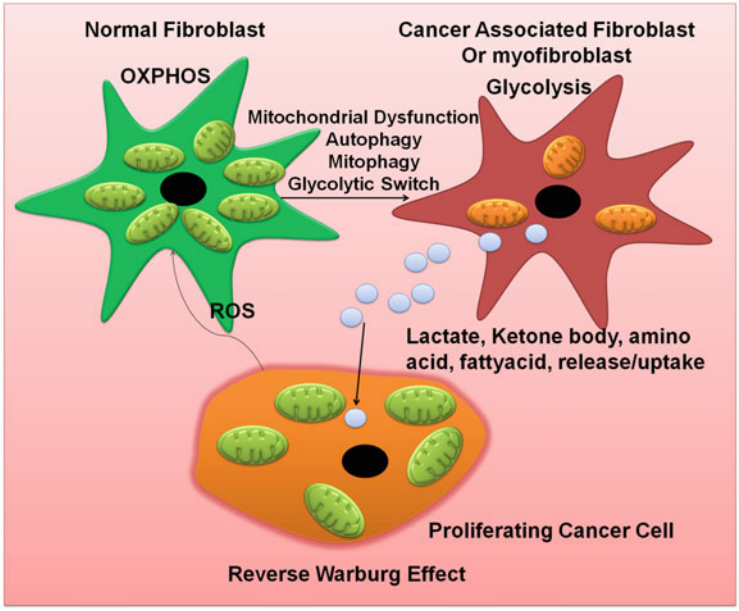

Fig. 6.1 Schematic representation of reverse Warburg effect. Diagram shows the mitochondrial dysfunction in fibroblast cells mediated by the ROS released by cancer cells leading to the up-regulation of aerobic glycolysis. The release of lactate and other high energy fuels by fibroblasts are then utilised by cancer cells for metabolic processes like OXPHOS

signalling pathways. It is also reported to aggravate oxidative stress mediated mitochondrial dysfunctions in CAFs (Sotgia et al. 2009; Witkiewicz et al. 2009, 2010). Furthermore, it is noticed that there is an elevated expression of mono-carboxylate transporters (MCTs) which performs the role of 'energy transfer device' (e.g., lactate) between CAFs and cancer cells through 'lactate shuttle' (Choi et al. 2013; Cirri and Chiarugi 2012; Rae et al. 2009; Whitaker-Menezes et al. 2011b; Witkiewicz et al. 2012). The reverse Warburg effect is basically the co-existence of metabolic alterations in both stromal cells and cancer cells depending on the demand of energy. On one hand, H_2O_2 secreted by cancer cells forms an oxidative microenvironment and up-regulate MCT1 to mediate enhance uptake of lactate. On the other hand, the stromal cells react to oxidative stress generated by H_2O_2 by mediating HIF-1-induced autophagic flux. This may lead to at least two levels of consequences. Firstly, it can mediate the degradation of Cav-1 by the autophagic process leading to tumor progression. Secondly, it can induce the transactivation of glycolytic enzymes by HIF-1 and up-regulation of MCT4 that mediates the efflux of lactate. Moreover, high expression of MCT4 in stromal cells is associated with a poor overall survival rate than low stromal Cav-1 status (Galluzzi et al. 2012). Again, loss of Cav-1 in stromal cells up-regulates the expression of glycolytic enzyme pyruvate kinase M2 (PKM2) and glycolysis. Cav-1 null stromal cells are also demonstrated to have acclerated lactate dehydrogenase-A (LDH-A) activity. Moreover, according to

documents an increased glycolysis pathway in stromal cells fuels the OXPHOS pathway in adjacent cancer cells (Capparelli et al. 2012). CAF cells when co-cultured with RAS-and NF-κB–dependent head and neck squamous cell carcinoma (HNSCC) cell line could trigger metabolic reprogramming via oxidative stress that brings about lactate shuttling with the help of MCT1/MCT4 to promote metabolic coupling between the tumor and tumor stroma (Curry et al. 2014). Along with the stromal and tumoral metabolic coupling, the lactate shuttle maintains an acid–base balance by inhibiting the generation of a fatal acidic microenvironment (Lee and Yoon 2015; Martinez-Outschoorn et al. 2011b). The tumor-derived lactate and autophagic fibroblasts derived lactate together perform additional roles. Lactates when taken up by endothelial cells via MCT1 stimulate autocrine signalling by NF-kB/IL-8 pathway to promote angiogenesis (Vegran et al. 2011). Lactate release via MCT4 from the breast and colon cancer cells are reported to induce IL-8-dependent angiogenesis (Azuma et al. 2007; Polet and Feron 2013). Again, lactate can also induce vascular endothelial growth factor-A (VEGFA) expression via HIF-1α activation (De Saedeleer et al. 2012; Lee et al. 2015; Sanità et al. 2014). The 'biofuel' lactate-induced generation of IL-8 and VEGFA together encourage pro-survival and pro-angiogenic activities in tumor growth (Polet and Feron 2013). Moreover, to fulfil the energetic demands, cancer cells use several nutrients including glucose, lactate, and glutamine. The schematic diagram of the molecular regulation of the reverse Warburg effect is represented in Fig. 6.2.

6.2.3 Two Compartment Model of Tumor Metabolism

A compartment-specific role of autophagy in tumor metabolism was proposed to explain the metabolic paradigm (Fig. 6.3). As discussed earlier, this model describes that occurance of autophagy, mitochondrial dysfunction, and mitophagy in tumor stroma results in the recycling of nutrients and provides chemical high-energy 'fuels,' and building blocks. This triggers the anabolic growth of tumors by inducing oxidative mitochondrial metabolism and autophagy mediated resistance in cancer cells. This stromal-epithelial metabolic coupling is popularly termed as the 'two-compartment tumor metabolism' (Martinez-Outschoorn et al. 2012; Salem et al. 2012). This hypothesis is stringently verified by experimenting on two genetic variants involving the fibroblasts with constitutive autophagic activity in addition to mitochondrial dysfunction and autophagy-resistant cancer cells with enhanced mitochondrial activity. Golgi phosphoprotein 3 (GOLPH3) overexpressing autophagy resistant cells are shown to display mitochondrial biogenesis. However, the damage-regulated autophagy modulator (DRAM) and liver kinase B1 (LKB1) overexpressing cells stimulated AMP-kinase activation and autophagy in CAFs (Salem et al. 2012). This autophagic fibroblasts exhibited mitochondrial dysfunction, increased glycolysis and generation of mitochondrial fuels. Both types of cells, that is, autophagic fibroblasts and autophagy resistant cancer cells promoted tumor growth where CAFs displayed glycolysis and cancer cell showed increased OXPHOS. In breast cancer, the activation of GPER/cAMP/

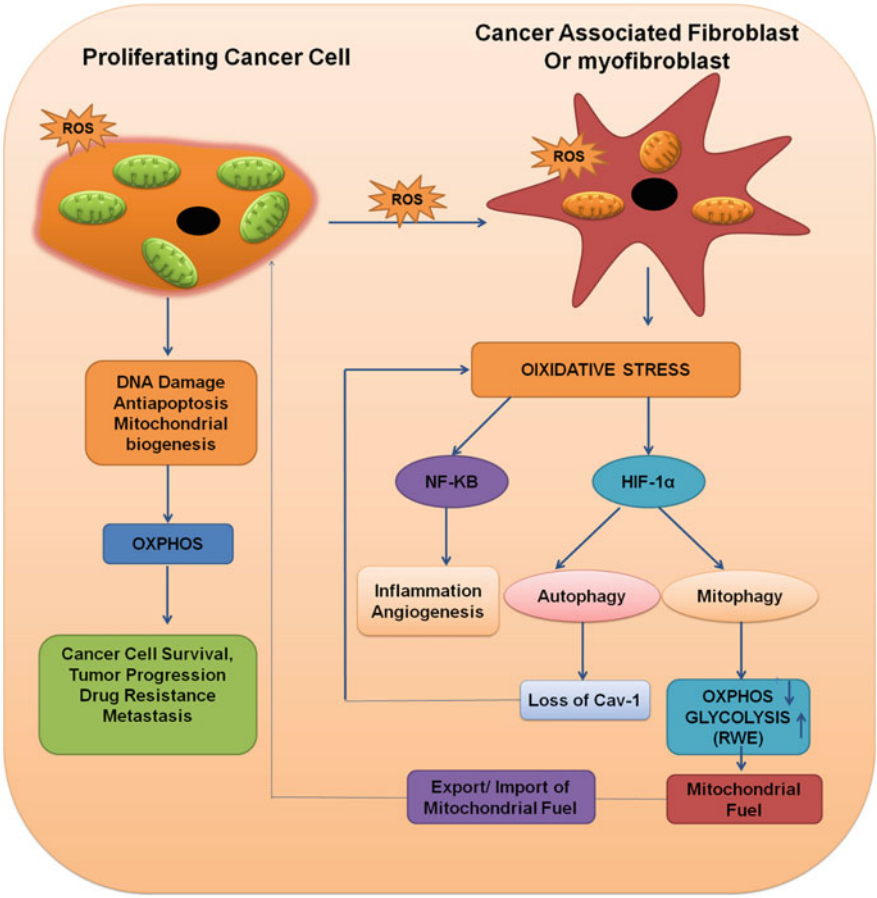

Fig. 6.2 Molecular mechanism of reverse Warburg effect. Oxidative stress generated in CAFs by ROS produced from cancer cells causes activation of autophagy/mitophagy and loss of Cav-1. Again, the stimulation of glycolysis occurs to release lactate which is then transported from CAFs to cancer cells to carry out OXPHOS

PKA/CREB and PI3K/AKT/mTORC1 signalling pathways in CAFs stimulate the aerobic glycolysis switch to secrete pyruvate and lactate for fuelling the OXPHOS in cancer cells (Yu et al. 2017). Moreover, 'Warburg-like' cancer metabolism and DNA damage response in tumor microenvironment share a strong association by activating the downstream signalling of DNA damage/repair target gene DRAM (Salem et al. 2013; Sotgia et al. 2013; Yang et al. 2016b). Cancer cells usually take advantage such resulting metabolites from the altered metabolism operating in CAFs. Moreover, higher expression of GLUT1 is seen in the fibroblasts cells when co-cultured with prostate cancer cells (Kihira et al. 2011; Sanita et al. 2014; Sun et al. 2014). Moreover, CAFs also exports lactate through MCT4 (Andersen et al. 2015; Sanità et al. 2014). Intriguingly, the prostate cancer cells on the other

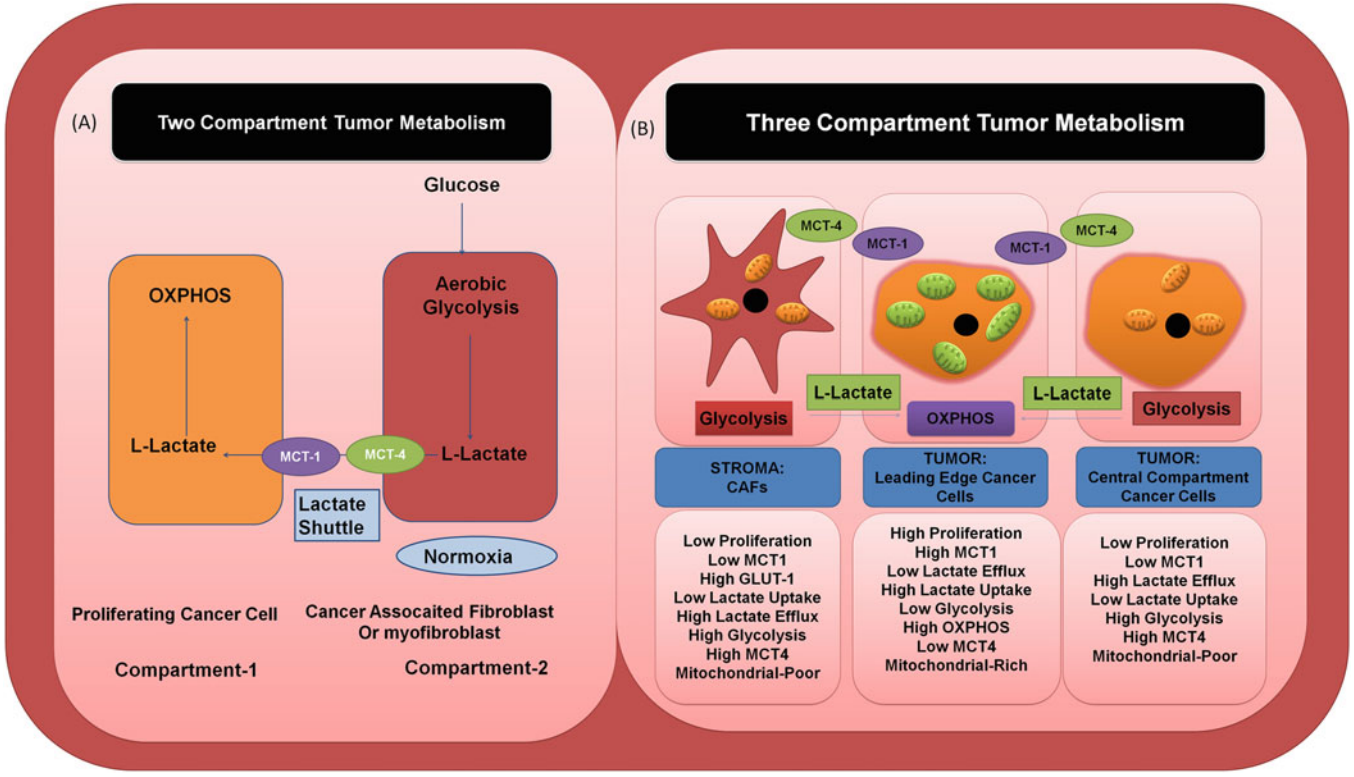

Fig. 6.3 (**a**) Two compartment and (**b**) three compartment tumor metabolism. The two-compartment tumor metabolism involves the metabolic coupling between cancer and cancer-associated fibroblast cells. The three-compartment tumor metabolism involves the cancer cells at edge, cancer cells at core and cancer-associated fibroblast cells

hand showed decreased GLUT1 expression and increased lactate influx via MCT1 (Fiaschi et al. 2012). Similar findings are also reported in breast cancer cells (Johnson et al. 2017; Le Floch et al. 2011; Witkiewicz et al. 2012). Furthermore, in another co-culture system with pancreatic cancer cells, MCT1 inhibition in CAFs is shown to decrease the expression pyruvate kinase 2 (PK2) along with the glucose import and lactate secretion (Giannoni et al. 2015). Consistent with the reverse Warburg effect, metastatic breast cancer cells could potentially amplify OXPHOS whereas the adjacent stromal cells were reported to lack detectable mitochondria and perform glycolysis. This was proved by double labelling experiments with the molecular marker for glycolysis (MCT4) and OXPHOS (TOMM20 or COX). This experimet discovered the presence of at least two distinct metabolic compartments that lie side-by-side both in primary tumors and their metastases (Sotgia et al. 2012). Again, there is little information about lipid metabolism in tumor microenvironment. Breast cancer cells in response to CAFs-conditioned media led to the overexpression of fatty acid transporter 1 (FATP1) and accumulation of lipid in cancer cells. Here, FATP1 negotiates the symbiosis of lipid metabolism between breast cancer cells and CAFs (Lopes-Coelho et al. 2018). CAFs are also shown to transport lysophospholipids (lsyo-PLs) directly to the pancreatic cancer cells via lipid droplets. Moreover, fibroblasts release lipids to the neighbouring cancers cells through microvesicles in melanoma and prostate cancer (Lopes-Coelho et al. 2018; Santi et al. 2015). Similar to lipid transport, glutamine release by CAFs and uptake by cancer cells show another aspect of metabolic coupling in tumor stroma. In ovarian cancer, glutamine metabolism in CAFs is reported to promote tumor growth with an increase in glutamine transporter SLC6A14 in cancer cells when co-cultured with CAFs. Thus, co-targeting the glutaminase in cancer and glutamine synthetase in CAFs will provide new insight into cancer therapeutics (Ko et al. 2011; Yang et al. 2016a).

6.2.4 Three Compartment Model of Tumor Metabolism

Reports also propose the presence of a three compartments model of tumor metabolism in cancer (Fig. 6.3). According to this model, the first metabolic compartment comprising cancer cells in periphery depends on OXPHOS. Whereas the second metabolic compartment consisting of cells occupying the deeper layer of tumors is often found to have more glycolytic (aerobic or anaerobic) activity. The third metabolic compartment comprising of fibroblast cells in tumor stroma undergoes aerobic glycolysis. Interestingly, this model is experimentally shown through elevated MCT4 expression in tumor stroma and deeper tumor to facilitate the release of biofuels. However, MCT1 expression level was found to be higher in the leading tumor edge. The OXPHOS execution in the leading tumor edge was confirmed by functional LDH-B and mitochondrial metabolism marker translocase of outer mitochondrial membrane 20 (TOMM20) (Curry et al. 2014). High oxidative stress ($MCT4^{+}$) is an important feature in cancer tissues as well as tumor stromal cells with higher tumor stage. High oxidative stress is also a marker for cancer-associated

fibroblasts and a key hallmark of cancer tissues which render them the ability to exploit the adjacent proliferating and mitochondrial-rich cancer cells. Two of the metabolic compartment that are the 'non-proliferating' populations of cells (Ki-67$^-$/MCT4$^+$) supply high-energy mitochondrial 'fuels' to the 'proliferative' cancer cells to catabolize and derive energy thereby play an essential role in determining the clinical outcome of the disease. In normal mucosa of head and neck cancers also, there are evidence of the presence of three-compartment metabolism. The normal basal stem cells are the proliferative (Ki-67$^+$), mitochondrial-rich (TOMM20$^+$/COX$^+$) and can have the ability to uptake mitochondrial fuel like L-lactate and ketone bodies (MCT1$^+$). It can be inferred that OXPHOS is a common characteristic of both normal stem cells and proliferating cancer cells (Fig. 6.4) (Curry et al. 2013). In head and neck cancer, similarly, a population of highly proliferative epithelial cancer cells with high mitochondrial content and ability for mitochondrial fuels import (Ki-67$^+$/TOMM20$^+$/COX$^+$/MCT1$^+$) were identified. Such proliferating cells are found to be surrounded by the non-proliferating epithelial tumor as well as stromal cells (Ki-67$^-$) that are deficient in mitochondria (TOMM20$^-$/COX$^-$/MCT1$^-$) and are displaying oxidative stress and glycolysis (MCT4$^+$). For simpler understanding it can be stated that 'Three compartment tumor metabolism' comprises (1) non-proliferative and mitochondrial-poor cancer cells (Ki-67$^-$/TOMM20$^-$/COX$^-$/MCT1$^-$); (2) proliferative and mitochondrial-rich cancer cells (Ki-67$^+$/TOMM20$^+$/COX$^+$/MCT1$^+$) and (3) non-proliferative and mitochondrial-poor stromal cells (Ki-67$^-$/TOMM20$^-$/COX$^-$/MCT1$^-$). The non-proliferative cancer along with the stromal cells offeres metabolites for OXPHOSto be operated in proliferating cancer cells (Bagordakis et al. 2016). Rapidly proliferating and poorly differentiated stem-cell-like HNSCC cancer cells have higher level of OXPHOS activity. Recently, Curry et al. documented a potential relationship of cancer stemness with lactate/ketone uptake and mitochondrial metabolism in head and neck cancer. Moreover, the three-compartment metabolism in HNSCC tumors involves the (1) hyper-proliferative (Ki-67$^+$), mitochondrial-rich (TOMM20$^+$/COX$^+$) and mitochondrial fuels import abled (MCT1$^+$) poorly differentiated cancer cells (CSCs) that undergo OXPHOS. Contrary to the proliferating cancer cells, the stromal cells and well-differentiated cancer cells are mitochondrial-poor, glycolytic and non-proliferative. The non-proliferating compartments of tumor are MCT4$^+$, that is, with characteristics like oxidative stress and mitochondrial dysfunction that can generate and export of L-lactate and ketone body (Curry et al. 2013) (Fig. 6.4). However, another category of cells, that are, cancer stem cells (CSCs) are too an important regulator of TME and tumorigenesis. Intriguingly, CSCs are documented to rely more on OXPHOS for their energy production. However, the CSCs also seem to be metabolically plastic i.e., they can exhibit both glycolytic/and oxidative phenotype (combined phenotype) depending on the demand. The quiescent and non-proliferative CSCs are OXPHOS phenotype with high mitochondrial mass whereas the proliferative CSCs show combined phenotype (Chae and Kim, 2018). Therefore, it is obvious that there is a multicompartmental metabolism in the tumor depending on the micro-environmental condition and demand for proliferation (Fig. 6.4).

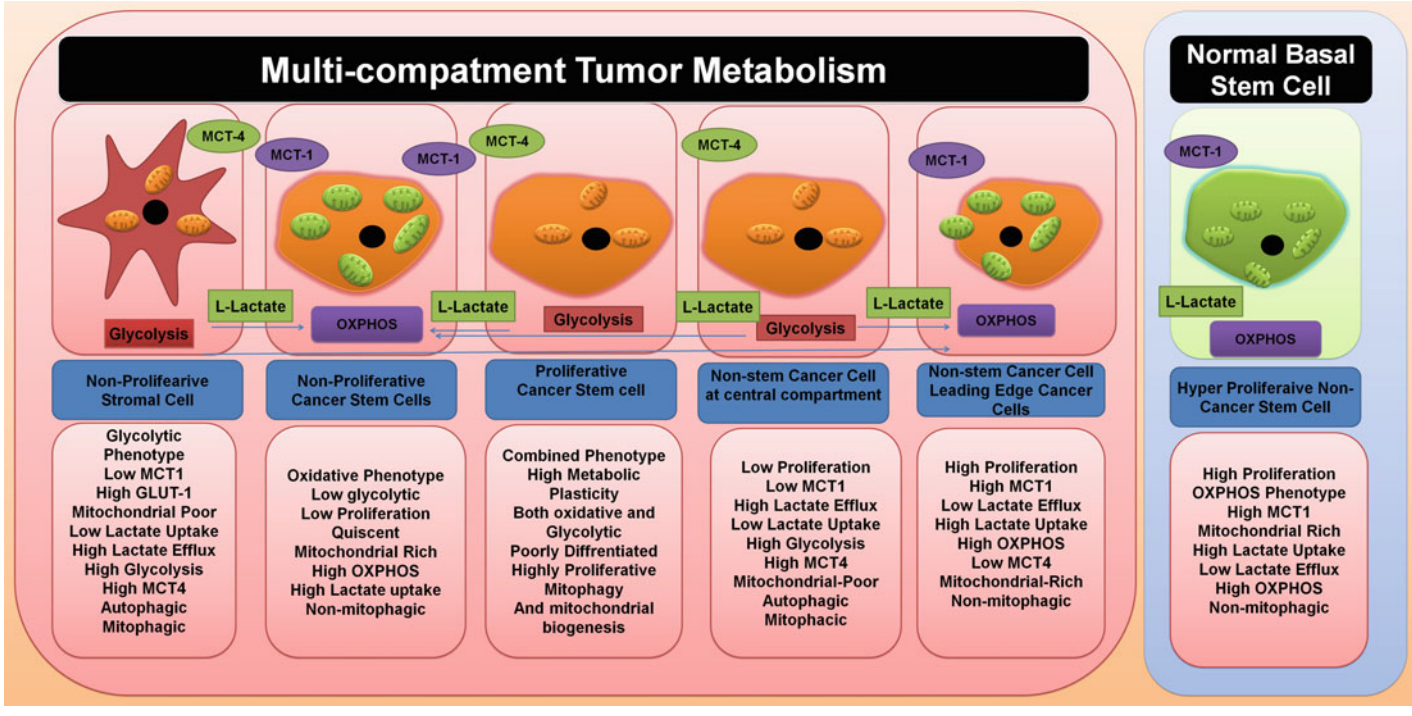

Fig. 6.4 Multi-compartment tumor metabolism. Figure displays the multiple compartments available in tumor stroma for metabolic coupling which comprises of nonstem cancer cells of edge, nonstem cancer cells of the core, non-proliferative cancer stem cells, proliferative cancer stem cells and cancer-associated fibroblasts

6.3 Mitophagy: The Regulator of Reverse Warburg Effect

Autophagy is basically an evolutionarily conserved catabolic process where a cell is programmed to self digest its cytoplasmic content to release metabolites and generate ATP during nutrient starvation, hypoxia, chemo/radio-therapeutic stress, and oncogene activation. Moreover, autophagy also provides a survival advantage where cancer cells are protected in response to metabolic deprivation and hypoxia during tumor progression. Unlike this bulk or non-selective autophagy, the selective autophagy or cargo-specific autophagy encourages removal of superfluous or damaged organelles and long-lived protein aggregates under nutrient-rich conditions. Lemasters et al. coined the term "mitophagy" to explain the autophagolysosomal degradation of mitochondria. Mitophagy maintains mitochondrial quality control and homeostasis in cells during normal cyto-physiological condition. Autophagic removal of dysfunctional mitochondria and metabolic turnover by mitophagy can promote cellular protection and chemoresistance in many cancers. Many reports are available to describe about the pro-tumor role of Parkin-dependent mitophagy as they regulate metabolism in tumor microenvironment (Naik et al. 2019). Occurrence of glycolysis and OXPHOS is directly related with the structural and functional dynamics of mitochondria. Healthy mitochondria can easily carry out OXPHOS while the dysfunctional ones cannot do so. Therefore, they must be eliminated from the cells via mitophagy. The elimination of dysfunctional mitochondria leads to the decrease in mitochondrial content forcing the cells to opt for alternative bioenergetics pathway like glycolysis. In order to exploit the fibroblast cells to release energy rich fuels, cancer cells must instigate mitophagy in CAFs According to Lisanti et al. stromal cells, such as fibroblasts lose their mitochondria by mitophagy to carry out the reverse Warburg Effect (Martinez-Outschoorn et al. 2010a). There is also translational evidence to support mitophagic tumor stroma of cancer metabolism. It was found that in the co-culture system of cancer cells and fibroblasts, the later showed remarkable alteration in mitochondrial content. Moreover, cancer cells are shown to display very low mitochondrial mass under homotypic culture conditions. However, when cultured with fibroblasts, there occurs a significant increase in the mitochondrial mass in cancer cells and a decrease in the mitochondrial mass in fibroblasts (Martinez-Outschoorn et al. 2010a, d). Mitophagy has to play a critical responsibilty in the generation of a glycolytic phenotype in cancer (Fig. 6.5). It is also important to mention that homotypic cancer cells when administered with lactate showed a considerable augmentation in mitochondrial content, indicating that administration of lactate phenocopies the presence of reactive fibroblasts with activated mitophagy. A unilateral transfer of energy takes place from glycolytic stromal fibroblast cells to oxidative cancer cells developing a parasitic relationship among them. Upon oxidative stress via the release of ROS by the cancer cells, Cav-1 is degraded (Sung et al. 2018). Interestingly, a study by Castello-Cros et al. showed that Cav-1 loss in stromal fibroblasts in mammary cells leads to overexpression of plasminogen activator inhibitor type 1 and 2 (PAI-1/2). PAI-1/2 overexpressing fibroblast cells upon co-culture with breast cancer cells could promote tumor growth and metastasis by inducing OXPHOS in nearby cancer cells.

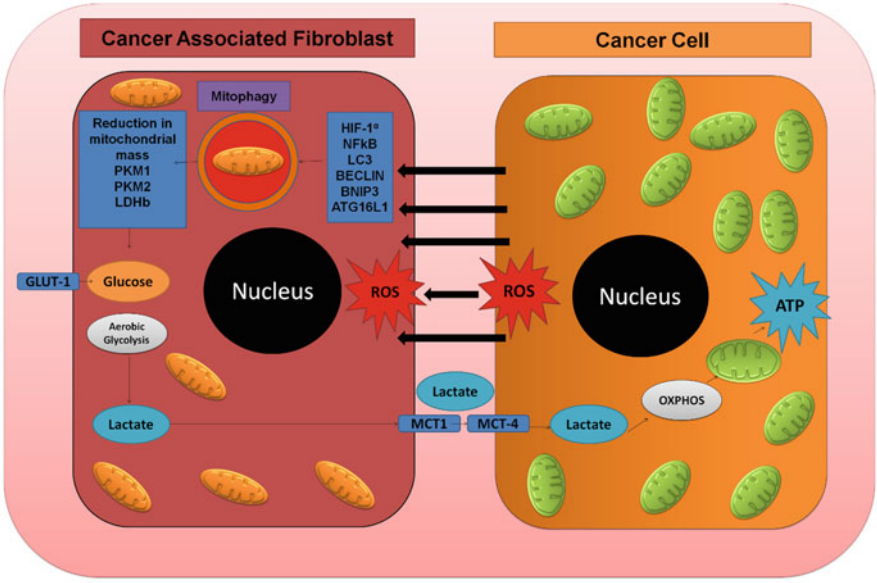

Fig. 6.5 Mitophagy regulating the reverse Warburg effect. Release of ROS to nearby CAFs causes mitochondrial dysfunction and activation HIF-1α, NF-kB, DRAM, LC3, BNIP3, BNIP3L, ATG16L1 and so on which further leads to the activation of autophagy as well as mitophagy. Mitophagy activation leads to the reduction of mitochondrial mass and OXPHOS. This also causes the stimulation of glycolysis in CAFs by the up-regulation of PKM1/2 and LDH-B expression. The lactate produced by aerobic glycolysis is then transported from CAF by MCT4 to cancer cells via the MCT1 to be used for OXPHOS

Moreover, it led to the up-regulation of activated fibroblasts markers such as calponin, vimentin, and fibronectin (Muda 2011). Subsequently, autophagy/mitophagy gets initiated in activated fibroblasts as evidenced from the overexpression of Beclin-1 and LAMP-1/2. Nextly, the ROS released by autophagic fibroblasts are reported to promote genomic instability in the cancer cells in the vicinity, thereby leading to the stimulation of further oncogenic mutations to support cancer cell proliferation. It can be summarized that the autophagic stroma model of cancer proposes the provocation of oxidative stress mediated mitochondrial dysfunction leading to the activation of autophagy/mitophagy process that finally helps in tumor invasion and metastasis. According to reports, as mitophagy occurs in the stromal fibroblast cells, they are compelled to perform aerobic glycolysis, resulting in the formation of excess lactate and/or ketones. One intriguing study involving the stable over-expression of autophagy and mitophagy gene BCL2/adenovirus E1B 19 kDa protein-interacting protein 3 (BNIP3), Cathepsin B (CTSB) and Autophagy-related protein 16-1 (ATG16L1) in telomerse-immortalised human fibroblasts (hTERT-BJ1) showed mitochondrial dysfunction, and constitutive autophagic/mitophagic features ensuing aerobic glycolysis to produce L-lactate and ketone bodies. Moreover, it was found that hypoxia-triggered break down of Cav-1

leads to the up-regulation of autophagy/mitophagy signature proteins such as microtubule-associated light chain 3 (LC3), ATG16L, BNIP3 and BCL2/adenovirus E1B 19 kDa protein-interacting protein 3-like (BNIP3L) (Martinez-Outschoorn et al. 2010c). Furthermore, knockdown of Cav-1 through si-RNA approach in stromal fibroblasts could enhance the level of lysosomal signature proteins and mitophagy markers. In another study, mammary fat pads of Cav-1 $^{(-/-)}$ null mice showed over-expression of autophagy/mitophagy markers like LC3 and BNIP3L (Qian et al. 2019; Thompson et al. 2012). Additionally, Cav-1 knockdown in fibroblasts was shown to promote ROS production, oxidative stress generation, and mitochondrial dysfunction which further led to the acceleration of autophagy/mitophagy. Moreover, in breast cancer patients, proteins like BNIP3L, PKM2 and LDH-B are expressed in high amount in Cav-1 deficient tumor stromal compartment (Lisanti et al. 2010). Furthermore, the expression of aerobic glycolysis marker PKM2, and LDH-B and mitophagy marker BNIP3L in CAFs are reported as effective biomarkers for the identification of high-risk cancer patients (Chiavarina et al. 2011; Martinez-Outschoorn et al. 2010d; Salem et al. 2012; Sung et al. 2020). Again, PKM1 has the ability to increase the glycolytic potential of stromal cells accompanied with the enhanced lactate output, whereas PKM2 can potentially induce the NF-kB-mediated autophagy induction and enhances the ketone body output. Such induction of oxidative stress-induced autophagy/mitophagy in the tumor stromal compartment offers a strategic mean to the cancer cells so that they can directly 'feed off' the nutrients, chemical building blocks, and energy-rich metabolites released by stromal fibroblasts (Chiavarina et al. 2011; Guido et al. 2012). This parasitic relationship and metabolic dependency also emphasizes a worthwhile solution to the 'autophagy paradox' in cancer aetiology and chemotherapy. Another document supporting this hypothesis reported the presence of a cytokine-mediated cross-talk between CAFs and cancer cells and a remarkable exchange of metabolites is noted among them. In this context, hypoxia-induced HIF-1α, cytokines, active ROS, and ammonia released by cancer cells in addition to the limited nutrient status in the tumor microenvironment are found to activate mitophagy and glycolysis in CAFs that culminates in the metabolic coupling through release of metabolites. As a result, anabolism is stimulated in cancer cells with downregulation of autophagy with consequent tumor growth (Sanita et al. 2014). The autophagy/mitophagy induction in hTERT (human telomerase reverse transcriptase)-immortalised fibroblasts as seen from the up-regulation of Beclin1, LAMP1, Cathepsin B, and BNIP3 is supposed to be mediated by DRAM genes. The DRAM overexpression in fibroblasts is also shown to downregulate the expression of Cav-1 and mitochondrial OXPHOS complexes indicating the onset of mitochondrial dysfunction and autophagy/mitophagy (Guido et al. 2012). Moreover, AMP–kinase activation also indicates the metabolic dysfunction in CAFs (Roy and Bera 2016). Moreover, under the up-regulated BNIP3 condition and DRAM overexpression, there was a decline in OXPHOS complexes I, III and IV suggesting the instigation of mitophagy onset (Liu et al. 2014; Salem et al. 2012). Again, any dysfunction in mitochondria triggers autophagy/mitophagy in CAFs that subsequently promotes reverse Warburg effect by agravating HIF-1α, and NF-kB. It

is also accompanied with the activation of antioxidant defence by encouraging the up-regulation of antioxidant enzymes (peroxiredoxin-1) and antiapoptotic proteins (TIGAR) (Martinez-Outschoorn et al. 2010c).

6.4 Conclusion and Future Perspective

Altered metabolism always provides the means to cancer cells to meet the need for unrestricted proliferation. Metabolic reprogramming of TME is highly necessary for tumor initiation and progression. Additionally, tumors are often seen consisting of a metabolically heterogeneous population where different cell types with different metabotypes coexist and collaborate to assure cancer progression. In TME, the CAFs represent a crucial cell type governing the metabolic crosstalk between cell types. Cancer cells have also developed the potential to use a variety of fuel sources. Tumor cells are shown to have increased aerobic as well as mitochondrial metabolism for ATP production, redox balance in various tumor cell types. Such metabolic alteration can be targeted for therapeutic intervention. The metabolic slavery of CAFs in TME can be a prospective target in this aspect. Strategies to inactivate CAFs myofibroblastic phenotype and disruption of metabolic crosstalk between tumor cells and CAFs might decrease the aggressiveness of tumor. In this regard, human patients are now experimented for various early-phase clinical trials (Kishton and Rathmell 2015; Ross and Critchlow 2014; Vander Heiden 2011). However, there are two most important concerns to be taken care of while adopting this approach. The first one is the metabolic plasticity adopted by cancer cells for allowing them to undergo rapid metabolic rewiring as a compensatory response. Secondly, the chance of developing potential toxicity in rapidly proliferating normal cells by targeting fundamental metabolic pathways. However, targeting metabolic pathways with anti-metabolites has been employed for a long time and serves as a successful treatment modality in multiple cancer types. Undoubtedly, insights into new metabolic models would lead to the development of novel biomarkers and parallel therapies which in turn would facilitate the discovery of personalised cancer medicine. As the oxidative stress and autophagy/mitophagy play a central role in this process, novel powerful antioxidants, autophagy/mitophagy inhibitors need to be developed to mitigate cancer.

References

Andersen S, Solstad Ø, Moi L et al (2015) Organized metabolic crime in prostate cancer: the coexpression of MCT1 in tumor and MCT4 in stroma is an independent prognosticator for biochemical failure. Urol Oncol 33:338.e9–338.e3.38E17. https://doi.org/10.1016/j.urolonc.2015.05.013

Astuti D, Latif F, Dallol A, Dahia PL, Douglas F, George E, Sköldberg F, Husebye ES, Eng C, Maher ER (2001) Gene mutations in the succinate dehydrogenase subunit SDHB cause susceptibility to familial pheochromocytoma and to familial paraganglioma. Am J Hum Genet 69:49–54

Avagliano A, Granato G, Ruocco MR, Romano V, Belviso I, Carfora A, Montagnani S, Arcucci A (2018) Metabolic Reprogramming of Cancer Associated Fibroblasts: The Slavery of Stromal Fibroblasts. Biomed Res Int 2018:6075403

Azuma M, Shi M, Danenberg KD, Gardner H, Barrett C, Jacques CJ, Sherod A, Iqbal S, El-Khoueiry A, Yang D (2007) Serum lactate dehydrogenase levels and glycolysis significantly correlate with tumor VEGFA and VEGFR expression in metastatic CRC patients. Pharmacogenomics 8:1705–1713

Bagordakis E, Sawazaki-Calone I, Macedo CC, Carnielli CM, de Oliveira CE, Rodrigues PC, Rangel AL, Dos Santos JN, Risteli J, Graner E, Salo T, Paes Leme AF, Coletta RD (2016) Secretome profiling of oral squamous cell carcinoma-associated fibroblasts reveals organization and disassembly of extracellular matrix and collagen metabolic process signatures. Tumour Biol 37:9045–9057

Bartrons R, Caro J (2007) Hypoxia, glucose metabolism and the Warburg's effect. J Bioenerg Biomembr 39:223–229

Baysal BE, Ferrell RE, Willett-Brozick JE, Lawrence EC, Myssiorek D, Bosch A, van der Mey A, Taschner PE, Rubinstein WS, Myers EN (2000) Mutations in SDHD, a mitochondrial complex II gene, in hereditary paraganglioma. Science 287:848–851

Bensaad K, Tsuruta A, Selak MA, Vidal MNC, Nakano K, Bartrons R, Gottlieb E, Vousden KH (2006) TIGAR, a p53-inducible regulator of glycolysis and apoptosis. Cell 126:107–120

Bonuccelli G, Tsirigos A, Whitaker-Menezes D, Pavlides S, Pestell RG, Chiavarina B, Frank PG, Flomenberg N, Howell A, Martinez-Outschoorn UE, Sotgia F, Lisanti MP (2010) Ketones and lactate "fuel" tumor growth and metastasis: Evidence that epithelial cancer cells use oxidative mitochondrial metabolism. Cell Cycle 9:3506–3514

Capparelli C, Guido C, Whitaker-Menezes D, Bonuccelli G, Balliet R, Pestell TG, Goldberg AF, Pestell RG, Howell A, Sneddon S (2012) Autophagy and senescence in cancer-associated fibroblasts metabolically supports tumor growth and metastasis, via glycolysis and ketone production. Cell Cycle 11:2285–2302

Chae YC, Kim JH (2018) Cancer stem cell metabolism: target for cancer therapy. BMB Rep 51 (7):319–326

Chang C-H, Qiu J, O'Sullivan D, Buck MD, Noguchi T, Curtis JD, Chen Q, Gindin M, Gubin MM, Van Der Windt GJ (2015) Metabolic competition in the tumor microenvironment is a driver of cancer progression. Cell 162:1229–1241

Chiavarina B, Whitaker-Menezes D, Martinez-Outschoorn UE, Witkiewicz AK, Birbe R, Howell A, Pestell RG, Smith J, Daniel R, Sotgia F, Lisanti MP (2011) Pyruvate kinase expression (PKM1 and PKM2) in cancer-associated fibroblasts drives stromal nutrient production and tumor growth. Cancer Biol Ther 12:1101–1113

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

Cirri P, Chiarugi P (2012) Cancer-associated-fibroblasts and tumour cells: a diabolic liaison driving cancer progression. Cancer Metastasis Rev 31:195–208

Curry JM, Tuluc M, Whitaker-Menezes D, Ames JA, Anantharaman A, Butera A, Leiby B, Cognetti DM, Sotgia F, Lisanti MP, Martinez-Outschoorn UE (2013) Cancer metabolism, stemness and tumor recurrence: MCT1 and MCT4 are functional biomarkers of metabolic symbiosis in head and neck cancer. Cell Cycle 12:1371–1384

Curry JM, Sprandio J, Cognetti D, Luginbuhl A, Bar-ad V, Pribitkin E, Tuluc M (2014) Tumor microenvironment in head and neck squamous cell carcinoma. Semin Oncol 41:217–234

Dang CV, J-w K, Gao P, Yustein J (2008) The interplay between MYC and HIF in cancer. Nat Rev Cancer 8:51–56

Dang L, White DW, Gross S, Bennett BD, Bittinger MA, Driggers EM, Fantin VR, Jang HG, Jin S, Keenan MC (2009) Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. Nature 462:739–744

De Saedeleer C, Copetti T, Porporato P, Verrax J, Feron O (2012) Lactate activates HIF-1 in oxidative but not in Warburg-phenotype human. PLoS One 7:e46571

DeBerardinis RJ, Mancuso A, Daikhin E, Nissim I, Yudkoff M, Wehrli S, Thompson CB (2007) Beyond aerobic glycolysis: transformed cells can engage in glutamine metabolism that exceeds the requirement for protein and nucleotide synthesis. Proc Natl Acad Sci 104:19345–19350

Egeblad M, Nakasone ES, Werb Z (2010) Tumors as organs: complex tissues tha interface with the entire organism. Dev Cell 18:884–901

Fantin VR, St-Pierre J, Leder P (2006) Attenuation of LDH-A expression uncovers a link between glycolysis, mitochondrial physiology, and tumor maintenance. Cancer Cell 9:425–434

Feron O (2009) Pyruvate into lactate and back: from the Warburg effect to symbiotic energy fuel exchange in cancer cells. Radiother Oncol 92:329–333

Fiaschi T, Marini A, Giannoni E, Taddei ML, Gandellini P, De Donatis A, Lanciotti M, Serni S, Cirri P, Chiarugi P (2012) Reciprocal metabolic reprogramming through lactate shuttle coordinately influences tumor-stroma interplay. Cancer Res 72:5130–5140

Friedl P, Alexander S (2011) Cancer invasion and the microenvironment: plasticity and reciprocity. Cell 147:992–1009

Galluzzi L, Kepp O, Kroemer G (2012) Reverse Warburg: straight to cancer. Cell Cycle 11:1059

Gao P, Tchernyshyov I, Chang T-C, Lee Y-S, Kita K, Ochi T, Zeller KI, De Marzo AM, Van Eyk JE, Mendell JT (2009) c-Myc suppression of miR-23a/b enhances mitochondrial glutaminase expression and glutamine metabolism. Nature 458:762–765

Giannoni E, Taddei ML, Morandi A, Comito G, Calvani M, Bianchini F, Richichi B, Raugei G, Wong N, Tang D (2015) Targeting stromal-induced pyruvate kinase M2 nuclear translocation impairs oxphos and prostate cancer metastatic spread. Oncotarget 6:24061

Gogvadze V, Zhivotovsky B, Orrenius S (2010) The Warburg effect and mitochondrial stability in cancer cells. Mol Asp Med 31:60–74

Gohil VM, Sheth SA, Nilsson R, Wojtovich AP, Lee JH, Perocchi F, Chen W, Clish CB, Ayata C, Brookes PS (2010) Nutrient-sensitized screening for drugs that shift energy metabolism from mitochondrial respiration to glycolysis. Nat Biotechnol 28:249–255

Guido C, Whitaker-Menezes D, Lin Z, Pestell RG, Howell A, Zimmers TA, Casimiro MC, Aquila S, Ando S, Martinez-Outschoorn UE, Sotgia F, Lisanti MP (2012) Mitochondrial fission induces glycolytic reprogramming in cancer-associated myofibroblasts, driving stromal lactate production, and early tumor growth. Oncotarget 3:798–810

Hanahan D, Coussens LM (2012) Accessories to the crime: functions of cells recruited to the tumor microenvironment. Cancer Cell 21:309–322

Hitosugi T, Kang S, Vander Heiden MG, Chung TW, Elf S, Lythgoe K, Dong S, Lonial S, Wang X, Chen GZ, Xie J, Gu TL, Polakiewicz RD, Roesel JL, Boggon TJ, Khuri FR, Gilliland DG, Cantley LC, Kaufman J, Chen J (2009) Tyrosine phosphorylation inhibits PKM2 to promote the Warburg effect and tumor growth. Sci Signal 2:ra73

Høyer-Hansen M, Jäättelä M (2007) AMP-activated protein kinase: a universal regulator of autophagy? Autophagy 3:381–383

Jiang E, Xu Z, Wang M, Yan T, Huang C, Zhou X, Liu Q, Wang L, Chen Y, Wang H, Liu K, Shao Z, Shang Z (2019) Tumoral microvesicle-activated glycometabolic reprogramming in fibroblasts promotes the progression of oral squamous cell carcinoma. FASEB J 33:5690–5703

Johnson JM, Cotzia P, Fratamico R, Mikkilineni L, Chen J, Colombo D, Mollaee M, Whitaker-Menezes D, Domingo-Vidal M, Lin Z (2017) MCT1 in invasive ductal carcinoma: monocarboxylate metabolism and aggressive breast cancer. Front Cell Dev Biol 5:27

Jose C, Bellance N, Rossignol R (2011) Choosing between glycolysis and oxidative phosphorylation: a tumor's dilemma? Biochim Biophys Acta Bioenerg 1807:552–561

Kihira Y, Yamano N, Izawa-Ishizawa Y, Ishizawa K, Ikeda Y, Tsuchiya K, Tamaki T, Tomita S (2011) Basic fibroblast growth factor regulates glucose metabolism through glucose transporter 1 induced by hypoxia-inducible factor-1α in adipocytes. Int J Biochem Cell Biol 43:1602–1611

Kishton RJ, Rathmell JC (2015) Novel therapeutic targets of tumor metabolism. Cancer J 21:62–69

Ko YH, Lin Z, Flomenberg N, Pestell RG, Howell A, Sotgia F, Lisanti MP, Martinez-Outschoorn UE (2011) Glutamine fuels a vicious cycle of autophagy in the tumor stroma and oxidative

mitochondrial metabolism in epithelial cancer cells: implications for preventing chemotherapy resistance. Cancer Biol Ther 12:1085–1097

Le Floch R, Chiche J, Marchiq I, Naiken T, Ilc K, Murray CM, Critchlow SE, Roux D, Simon M-P, Pouysségur J (2011) CD147 subunit of lactate/H+ symporters MCT1 and hypoxia-inducible MCT4 is critical for energetics and growth of glycolytic tumors. Proc Natl Acad Sci 108:16663–16668

Lee M, Yoon JH (2015) Metabolic interplay between glycolysis and mitochondrial oxidation: the reverse Warburg effect and its therapeutic implication. World J Biol Chem 6:148–161

Lee DC, Sohn HA, Park Z-Y, Oh S, Kang YK, Lee K-m, Kang M, Jang YJ, Yang S-J, Hong YK (2015) A lactate-induced response to hypoxia. Cell 161:595–609

Lisanti MP, Martinez-Outschoorn UE, Chiavarina B, Pavlides S, Whitaker-Menezes D, Tsirigos A, Witkiewicz AK, Lin Z, Balliet RM, Howell A, Sotgia F (2010) Understanding the "lethal" drivers of tumor-stroma co-evolution: emerging role(s) for hypoxia, oxidative stress and autophagy/mitophagy in the tumor micro-environment. Cancer Biol Ther 10:537–542

Liu K, Shi Y, Guo XH, Ouyang YB, Wang SS, Liu DJ, Wang AN, Li N, Chen DX (2014) Phosphorylated AKT inhibits the apoptosis induced by DRAM-mediated mitophagy in hepatocellular carcinoma by preventing the translocation of DRAM to mitochondria. Cell Death Dis 5: e1078

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

Maddocks OD, Vousden KH (2011) Metabolic regulation by p53. J Mol Med 89:237–245

Marroquin LD, Hynes J, Dykens JA, Jamieson JD, Will Y (2007) Circumventing the Crabtree effect: replacing media glucose with galactose increases susceptibility of HepG2 cells to mitochondrial toxicants. Toxicol Sci 97:539–547

Martinez-Outschoorn UE, Balliet RM, Rivadeneira DB, Chiavarina B, Pavlides S, Wang C, Whitaker-Menezes D, Daumer KM, Lin Z, Witkiewicz AK, Flomenberg N, Howell A, Pestell RG, Knudsen ES, Sotgia F, Lisanti MP (2010a) Oxidative stress in cancer associated fibroblasts drives tumor-stroma co-evolution: a new paradigm for understanding tumor metabolism, the field effect and genomic instability in cancer cells. Cell Cycle 9:3256–3276

Martinez-Outschoorn UE, Pavlides S, Whitaker-Menezes D, Daumer KM, Milliman JN, Chiavarina B, Migneco G, Witkiewicz AK, Martinez-Cantarin MP, Flomenberg N, Howell A, Pestell RG, Lisanti MP, Sotgia F (2010b) Tumor cells induce the cancer associated fibroblast phenotype via caveolin-1 degradation: implications for breast cancer and DCIS therapy with autophagy inhibitors. Cell Cycle 9:2423–2433

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 (2010c) Autophagy in cancer associated fibroblasts promotes tumor cell survival: role of hypoxia, HIF1 induction and NFkappaB activation in the tumor stromal microenvironment. Cell Cycle 9:3515–3533

Martinez-Outschoorn UE, Whitaker-Menezes D, Pavlides S, Chiavarina B, Bonuccelli G, Casey T, Tsirigos A, Migneco G, Witkiewicz A, Balliet R, Mercier I, Wang C, Flomenberg N, Howell A, Lin Z, Caro J, Pestell RG, Sotgia F, Lisanti MP (2010d) The autophagic tumor stroma model of cancer or "battery-operated tumor growth": a simple solution to the autophagy paradox. Cell Cycle 9:4297–4306

Martinez-Outschoorn UE, Lin Z, Trimmer C, Flomenberg N, Wang C, Pavlides S, Pestell RG, Howell A, Sotgia F, Lisanti MP (2011a) Cancer cells metabolically "fertilize" the tumor microenvironment with hydrogen peroxide, driving the Warburg effect: implications for PET imaging of human tumors. Cell Cycle 10:2504–2520

Martinez-Outschoorn UE, Pavlides S, Howell A, Pestell RG, Tanowitz HB, Sotgia F, Lisanti MP (2011b) Stromal–epithelial metabolic coupling in cancer: integrating autophagy and metabolism in the tumor microenvironment. Int J Biochem Cell Biol 43:1045–1051

Martinez-Outschoorn UE, Balliet RM, Lin Z, Whitaker-Menezes D, Howell A, Sotgia F, Lisanti MP (2012) Hereditary ovarian cancer and two-compartment tumor metabolism: epithelial loss

of BRCA1 induces hydrogen peroxide production, driving oxidative stress and NFκB activation in the tumor stroma. Cell Cycle 11:4152–4166

Martinez-Outschoorn UE, Curry JM, Ko YH, Lin Z, Tuluc M, Cognetti D, Birbe RC, Pribitkin E, Bombonati A, Pestell RG, Howell A, Sotgia F, Lisanti MP (2013) Oncogenes and inflammation rewire host energy metabolism in the tumor microenvironment: RAS and NFkappaB target stromal MCT4. Cell Cycle 12:2580–2597

Matoba S, Kang J-G, Patino WD, Wragg A, Boehm M, Gavrilova O, Hurley PJ, Bunz F, Hwang PM (2006) p53 regulates mitochondrial respiration. Science 312:1650–1653

Muda M (2011) DUSPs strike again: Comment on: Kozarova A, et al. Cell Cycle 2011; 10: 1669–78. Cell Cycle 10:2827–2835

Naik PP, Birbrair A, Bhutia SK (2019) Mitophagy-driven metabolic switch reprograms stem cell fate. Cell Mol Life Sci 76:27–43

Nilsson LM, Forshell TZP, Rimpi S, Kreutzer C, Pretsch W, Bornkamm GW, Nilsson JA (2012) Mouse genetics suggests cell-context dependency for Myc-regulated metabolic enzymes during tumorigenesis. PLoS Genet 8:e1002573

Obre E, Rossignol R (2015) Emerging concepts in bioenergetics and cancer research: metabolic flexibility, coupling, symbiosis, switch, oxidative tumors, metabolic remodeling, signaling and bioenergetic therapy. Int J Biochem Cell Biol 59:167–181

Osthus RC, Shim H, Kim S, Li Q, Reddy R, Mukherjee M, Xu Y, Wonsey D, Lee LA, Dang CV (2000) Deregulation of glucose transporter 1 and glycolytic gene expression by c-Myc. J Biol Chem 275:21797–21800

Palaskas N, Larson SM, Schultz N, Komisopoulou E, Wong J, Rohle D, Campos C, Yannuzzi N, Osborne JR, Linkov I (2011) 18F-fluorodeoxy-glucose positron emission tomography marks MYC-overexpressing human basal-like breast cancers. Cancer Res 71:5164–5174

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 (2009) The reverse Warburg effect: aerobic glycolysis in cancer associated fibroblasts and the tumor stroma. Cell Cycle 8:3984–4001

Pavlides S, Tsirigos A, Migneco G, Whitaker-Menezes D, Chiavarina B, Flomenberg N, Frank PG, Casimiro MC, Wang C, Pestell RG (2010) The autophagic tumor stroma model of cancer: Role of oxidative stress and ketone production in fueling tumor cell metabolism. Cell Cycle 9:3485–3505

Pavlides S, Vera I, Gandara R, Sneddon S, Pestell RG, Mercier I, Martinez-Outschoorn UE, Whitaker-Menezes D, Howell A, Sotgia F (2012) Warburg meets autophagy: cancer-associated fibroblasts accelerate tumor growth and metastasis via oxidative stress, mitophagy, and aerobic glycolysis. Antioxid Redox Signal 16:1264–1284

Pfeiffer T, Schuster S, Bonhoeffer S (2001) Cooperation and competition in the evolution of ATP-producing pathways. Science 292:504–507

Polet F, Feron O (2013) Endothelial cell metabolism and tumour angiogenesis: glucose and glutamine as essential fuels and lactate as the driving force. J Intern Med 273:156–165

Porporato PE, Dhup S, Dadhich RK, Copetti T, Sonveaux P (2011) Anticancer targets in the glycolytic metabolism of tumors: a comprehensive review. Front Pharmacol 2:49

Potter M, Newport E, Morten KJ (2016) The Warburg effect: 80 years on. Biochem Soc Trans 44:1499–1505

Qian XL, Pan YH, Huang QY, Shi YB, Huang QY, Hu ZZ, Xiong LX (2019) Caveolin-1: a multifaceted driver of breast cancer progression and its application in clinical treatment. Onco Targets Ther 12:1539–1552

Rae C, Nasrallah FA, Broer S (2009) Metabolic effects of blocking lactate transport in brain cortical tissue slices using an inhibitor specific to MCT1 and MCT2. Neurochem Res 34:1783–1791

Robey RB, Hay N (2009) Is Akt the "Warburg kinase"?-Akt-energy metabolism interactions and oncogenesis. Semin Cancer Biol 19:25–31

Ross SJ, Critchlow SE (2014) Emerging approaches to target tumor metabolism. Curr Opin Pharmacol 17:22–29

Roy A, Bera S (2016) CAF cellular glycolysis: linking cancer cells with the microenvironment. Tumour Biol 37:8503–8514

Salem AF, Whitaker-Menezes D, Lin Z, Martinez-Outschoorn UE, Tanowitz HB, Al-Zoubi MS, Howell A, Pestell RG, Sotgia F, Lisanti MP (2012) Twocompartment tumor metabolism: autophagy in the tumor microenvironment and oxidative mitochondrial metabolism (OXPHOS) in cancer cells. Cell Cycle 11:2545–2556

Salem AF, Al-Zoubi MS, Whitaker-Menezes D, Martinez-Outschoorn UE, Lamb R, Hulit J, Howell A, Gandara R, Sartini M, Galbiati F (2013) Cigarette smoke metabolically promotes cancer, via autophagy and premature aging in the host stromal microenvironment. Cell Cycle 12:818–825

Sandulache VC, Ow TJ, Pickering CR, Frederick MJ, Zhou G, Fokt I, Davis-Malesevich M, Priebe W, Myers JN (2011) Glucose, not glutamine, is the dominant energy source required for proliferation and survival of head and neck squamous carcinoma cells. Cancer 117:2926–2938

Sanita P, Capulli M, Teti A, Galatioto GP, Vicentini C, Chiarugi P, Bologna M, Angelucci A (2014) Tumor-stroma metabolic relationship based on lactate shuttle can sustain prostate cancer progression. BMC Cancer 14:154

Sanità P, Capulli M, Teti A, Galatioto GP, Vicentini C, Chiarugi P, Bologna M, Angelucci A (2014) Tumor-stroma metabolic relationship based on lactate shuttle can sustain prostate cancer progression. BMC Cancer 14:154

Santi A, Caselli A, Ranaldi F, Paoli P, Mugnaioni C, Michelucci E, Cirri P (2015) Cancer associated fibroblasts transfer lipids and proteins to cancer cells through cargo vesicles supporting tumor growth. Biochim Biophys Acta 1853:3211–3223

Semenza GL (2010) HIF-1: upstream and downstream of cancer metabolism. Curr Opin Genet Dev 20:51–56

Shim H, Dolde C, Lewis BC, Wu C-S, Dang G, Jungmann RA, Dalla-Favera R, Dang CV (1997) c-Myc transactivation of LDH-A: implications for tumor metabolism and growth. Proc Natl Acad Sci 94:6658–6663

Slavov N, Budnik BA, Schwab D, Airoldi EM, van Oudenaarden A (2014) Constant growth rate can be supported by decreasing energy flux and increasing aerobic glycolysis. Cell Rep 7:705–714

Sonveaux P, Vegran F, Schroeder T, Wergin MC, Verrax J, Rabbani ZN, De Saedeleer CJ, Kennedy KM, Diepart C, Jordan BF, Kelley MJ, Gallez B, Wahl ML, Feron O, Dewhirst MW (2008) Targeting lactate-fueled respiration selectively kills hypoxic tumor cells in mice. J Clin Invest 118:3930–3942

Sotgia F, Del Galdo F, Casimiro MC, Bonuccelli G, Mercier I, Whitaker-Menezes D, Daumer KM, Zhou J, Wang C, Katiyar S, Xu H, Bosco E, Quong AA, Aronow B, Witkiewicz AK, Minetti C, Frank PG, Jimenez SA, Knudsen ES, Pestell RG, Lisanti MP (2009) Caveolin-1−/− null mammary stromal fibroblasts share characteristics with human breast cancer-associated fibroblasts. Am J Pathol 174:746–761

Sotgia F, Whitaker-Menezes D, Martinez-Outschoorn UE, Flomenberg N, Birbe RC, Witkiewicz AK, Howell A, Philp NJ, Pestell RG, Lisanti MP (2012) Mitochondrial metabolism in cancer metastasis: visualizing tumor cell mitochondria and the "reverse Warburg effect" in positive lymph node tissue. Cell Cycle 11:1445–1454

Sotgia F, Martinez-Outschoorn UE, Lisanti MP (2013) Cancer metabolism: new validated targets for drug discovery. Oncotarget 4:1309

Sun P, Hu J-W, Xiong W-J, Mi J (2014) miR-186 regulates glycolysis through Glut1 during the formation of cancer-associated fibroblasts. Asian Pac J Cancer Prev 15:4245–4250

Sung JS, Kang S, Lee JH, Mun SG, Kim BG, Cho NH (2018) Integrin beta4-induced mitophagy promotes the lactate production of cancer-associated fibroblasts in breast cancer. In: AACR

Sung JS, Kang CW, Kang S, Jang Y, Chae YC, Kim BG, Cho NH (2020) ITGB4-mediated metabolic reprogramming of cancer-associated fibroblasts. Oncogene 39:664–676

Thompson DE, Siwicky MD, Moorehead RA (2012) Caveolin-1 expression is elevated in claudin-low mammary tumor cells. Cancer Cell Int 12:6

Vadlakonda L, Dash A, Pasupuleti M, Anil Kumar K, Reddanna P (2013) Did we get Pasteur, Warburg, and Crabtree on a right note? Front Oncol 3:186

Vander Heiden MG (2011) Targeting cancer metabolism: a therapeutic window opens. Nat Rev Drug Discov 10:671–684

Vegran F, Boidot R, Sonveaux P, Feron O (2011) 1004 ORAL lactate influx and efflux through monocarboxylate transporters bridge cancer cell metabolism and angiogenesis. Eur J Cancer 47: S98

Villena JA, Kralli A (2008) ERRalpha: a metabolic function for the oldest orphan. Trends Endocrinol Metab 19:269–276

Vincent AS, Phan TT, Mukhopadhyay A, Lim HY, Halliwell B, Wong KP (2008) Human skin keloid fibroblasts display bioenergetics of cancer cells. J Invest Dermatol 128:702–709

Warburg O (1925) The metabolism of carcinoma cells. J Cancer Res 9:148–163

Warburg O (1956) On the origin of cancer cells. Science 123:309–314

Whitaker-Menezes D, Martinez-Outschoorn UE, Flomenberg N, Birbe R, Witkiewicz AK, Howell A, Pavlides S, Tsirigos A, Ertel A, Pestell RG (2011a) Hyperactivation of oxidative mitochondrial metabolism in epithelial cancer cells in situ: visualizing the therapeutic effects of metformin in tumor tissue. Cell Cycle 10:4047–4064

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 (2011b) Evidence for a stromal-epithelial "lactate shuttle" in human tumors: MCT4 is a marker of oxidative stress in cancer-associated fibroblasts. Cell Cycle 10:1772–1783

Wilde L, Roche M, Domingo-Vidal M, Tanson K, Philp N, Curry J, Martinez-Outschoorn U (2017) Metabolic coupling and the Reverse Warburg Effect in cancer: Implications for novel biomarker and anticancer agent development. Semin Oncol 44:198–203

Witkiewicz AK, Dasgupta A, Nguyen KH, Liu C, Kovatich AJ, Schwartz GF, Pestell RG, Sotgia F, Rui H, Lisanti MP (2009) Stromal caveolin-1 levels predict early DCIS progression to invasive breast cancer. Cancer Biol Ther 8:1071–1079

Witkiewicz AK, Dasgupta A, Sammons S, Er O, Potoczek MB, Guiles F, Sotgia F, Brody JR, Mitchell EP, Lisanti MP (2010) Loss of stromal caveolin-1 expression predicts poor clinical outcome in triple negative and basal-like breast cancers. Cancer Biol Ther 10:135–143

Witkiewicz AK, Whitaker-Menezes D, Dasgupta A, Philp NJ, Lin Z, Gandara R, Sneddon S, Martinez-Outschoorn UE, Sotgia F, Lisanti MP (2012) Using the "reverse Warburg effect" to identify high-risk breast cancer patients: stromal MCT4 predicts poor clinical outcome in triple-negative breast cancers. Cell Cycle 11:1108–1117

Yan H, Bigner DD, Velculescu V, Parsons DW (2009) Mutant metabolic enzymes are at the origin of gliomas. Cancer Res 69:9157–9159

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 (2016a) Targeting stromal glutamine synthetase in tumors disrupts tumor microenvironment-regulated cancer cell growth. Cell Metab 24:685–700

Yang X, Xu X, Zhu J, Zhang S, Wu Y, Wu Y, Zhao K, Xing C, Cao J, Zhu H (2016b) miR-31 affects colorectal cancer cells by inhibiting autophagy in cancer-associated fibroblasts. Oncotarget 7:79617

Yeung S, Pan J, Lee M-H (2008) Roles of p53, MYC and HIF-1 in regulating glycolysis—the seventh hallmark of cancer. Cell Mol Life Sci 65:3981

Yu T, Yang G, Hou Y, Tang X, Wu C, Wu X, Guo L, Zhu Q, Luo H, Du Y (2017) Cytoplasmic GPER translocation in cancer-associated fibroblasts mediates cAMP/PKA/CREB/glycolytic axis to confer tumor cells with multidrug resistance. Oncogene 36:2131–2145

Zhang XH-F, Jin X, Malladi S, Zou Y, Wen YH, Brogi E, Smid M, Foekens JA, Massagué J (2013) Selection of bone metastasis seeds by mesenchymal signals in the primary tumor stroma. Cell 154:1060–1073

Zhang G, Li J, Wang X, Ma Y, Yin X, Wang F, Zheng H, Duan X, Postel GC, Li X-F (2015) The reverse Warburg effect and 18F-FDG uptake in non–small cell lung cancer A549 in mice: a pilot study. J Nucl Med 56:607–612

Zheng M, Wang Y-H, Wu X-N, Wu S-Q, Lu B-J, Dong M-Q, Zhang H, Sun P, Lin S-C, Guan K-L (2011) Inactivation of Rheb by PRAK-mediated phosphorylation is essential for energy-depletion-induced suppression of mTORC1. Nat Cell Biol 13:263–272

Zong WX, Rabinowitz JD, White E (2016) Mitochondria and cancer. Mol Cell 61:667–676