

REVIEW

# Understanding the Warburg effect and the prognostic value of stromal caveolin-1 as a marker of a lethal tumor microenvironment

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## Abstract

Cancer cells show a broad spectrum of bioenergetic states, with some cells using aerobic glycolysis while others rely on oxidative phosphorylation as their main source of energy. In addition, there is mounting evidence that metabolic coupling occurs in aggressive tumors, between epithelial cancer cells and the stromal compartment, and between well-oxygenated and hypoxic compartments. We recently showed that oxidative stress in the tumor stroma, due to aerobic glycolysis and mitochondrial dysfunction, is important for cancer cell mutagenesis and tumor progression. More specifically, increased autophagy/mitophagy in the tumor stroma drives a form of parasitic epithelial-stromal metabolic coupling. These findings explain why it is effective to treat tumors with either inducers or inhibitors of autophagy, as both would disrupt this energetic coupling. We also discuss evidence that glutamine addiction in cancer cells produces ammonia via oxidative mitochondrial metabolism. Ammonia production in cancer cells, in turn, could then help maintain autophagy in the tumor stromal compartment. In this vicious cycle, the initial glutamine provided to cancer cells would be produced by autophagy in the tumor stroma. Thus, we believe that parasitic epithelial-stromal metabolic coupling has important implications for cancer diagnosis and therapy, for example, in designing novel metabolic imaging techniques and establishing new targeted therapies. In direct support of this notion, we identified a loss of stromal caveolin-1 as a marker of oxidative stress, hypoxia, and autophagy in the tumor microenvironment, explaining its powerful predictive value. Loss of stromal caveolin-1 in breast cancers is associated with early tumor recurrence, metastasis, and drug resistance, leading to poor clinical outcome.

## The conventional 'Warburg effect' versus oxidative mitochondrial metabolism

The Warburg effect, also known as aerobic glycolysis, is defined as the propensity of cancer cells to take up high levels of glucose and to secrete lactate in the presence of oxygen. Warburg's original work indicated that while glucose uptake and lactate production are greatly elevated, a cancer cell's rate of mitochondrial respiration is similar to that of normal cells [1,2]. He, however, described it as a 'respiratory impairment' due to the fact that, in cancer cells, mitochondrial respiration is smaller, relative to their glycolytic power, but not smaller relative

to normal cells. He recognized that oxygen consumption is not diminished in tumor cells, but that respiration is disturbed because glycolysis persists in the presence of oxygen [1,2]. Unfortunately, the perception of his original findings was simplified over the years, and most subsequent papers validated that cancer cells undergo aerobic glycolysis and produce lactate, but did not measure mitochondrial respiration, and just presumed decreased tricarboxylic acid (TCA) cycle activity and reduced oxidative phosphorylation [1,2]. It is indeed well documented that, as a consequence of intra-tumoral hypoxia, the hypoxia-inducible factor (HIF)1 $\alpha$  pathway is activated in many tumors cells, resulting in the direct up-regulation of lactate dehydrogenase (LDH) and increased glucose consumption. For updated reviews on the Warburg effect, the reader is encouraged to refer to the following papers [3,4].

However, new findings compel us to reconsider the current model of cancer cell metabolism. First, not all tumors are associated with increased aerobic glycolysis,

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and in fact it is now clear that cancer cells utilize both glycolysis and oxidative phosphorylation to satisfy their metabolic needs. Experimental assessments of ATP production in cancer cells have demonstrated that oxidative pathways play a significant role in energy generation, and may be responsible for about 50 to 80% of the ATP generated [5-8]. Also, it should be considered that most studies were performed using isolated cancer cells, which may behave very differently from cancer cells *in vivo*, surrounded by their natural microenvironment [9] (see also Koukourakis and colleagues [10] for another viewpoint).

Second, several studies now clearly indicate that mitochondrial activity and oxidative phosphorylation support tumor growth. Loss-of-function mutations in the TCA cycle gene *IDH1* (isocitrate dehydrogenase 1) are found in about 70% of gliomas, but, interestingly, correlate with a better prognosis and improved survival, suggesting that severely decreased activity in one of the TCA cycle enzymes does not favor tumor aggressiveness [11]. The mitochondrial protein p32 was shown to maintain high levels of oxidative phosphorylation in human cancer cells and to sustain tumorigenicity *in vivo* [12]. In addition, STAT3 is known to enhance tumor growth and to predict poor prognosis in human cancers [13]. Interestingly, a pool of STAT3 localizes to the mitochondria, to sustain high levels of mitochondrial respiration [14] and to augment transformation by oncogenic Ras [15,16]. Similarly, the mitochondrial transcription factor A (TFAM), which is required for mitochondrial DNA replication and oxidative phosphorylation, is also required for K-Ras-induced lung tumorigenesis [17]. Finally, when constrained to use glycolysis by depletion of mitochondrial DNA, melanoma B16 cells (B16 $\rho^0$ ) and breast cancer T47D cells (T47D $\rho^0$ ), show severe impairment of tumorigenicity *in vivo* [18,19].

There is also evidence that pro-oncogenic molecules regulate mitochondrial function. Cyclin D1 inhibits mitochondrial function in breast cancer cells [20]. Overexpression of cyclin D1 is observed in about 50% of invasive breast cancers and is associated with a good clinical outcome [21], indicating that inhibition of mitochondrial activity correlates with favorable prognosis. Importantly, it was shown that the oncogene c-Myc stimulates mitochondrial biogenesis, and enhances glutamine metabolism by regulating the expression of mitochondrial glutaminase, the first enzyme in the glutamine utilization pathway [22]. Glutamine is an essential metabolic fuel that is converted to alpha-ketoglutarate and serves as a substrate for the TCA cycle or for glutathione synthesis, to promote energy production and cellular biosynthesis, and to protect against oxidative stress [23]. Interestingly, pharmacological targeting of mitochondrial glutaminase inhibits cancer cell transforming activity, suggesting that glutamine metabolism

and its role in fueling and replenishing the TCA cycle are required for neoplastic transformation [24] (for a recent review on glutamine cancer metabolism, see [2]).

### The reverse Warburg effect

It is increasingly apparent that the tumor microenvironment regulates neoplastic growth and progression. Activation of the stroma is a critical step required for tumor formation. Among the stromal players, cancer associated fibroblasts (CAFs) have recently taken center stage [25]. CAFs are activated, contractile fibroblasts that display features of myo-fibroblasts, express muscle-specific actin, and show an increased ability to secrete and remodel the extracellular matrix [26,27]. They are not just neutral spectators, but actively support malignant transformation [28] and metastasis [29], as compared to normal resting fibroblasts. For example, in a humanized mouse model of breast cancer, oncogenically driven human organoids develop tumors only if co-injected with immortalized fibroblasts and not with normal primary fibroblasts, suggesting that stromal activation is required for breast cancer formation [30].

Importantly, the tumor stroma dictates clinical outcome and constitutes a source of potential biomarkers [31]. Expression profiling has identified a cancer-associated stromal signature that predicts good and poor clinical prognosis in breast cancer patients, independently of other factors [32-34].

We and others have recently shown that a loss of caveolin-1 (Cav-1) in the stromal compartment is a novel biomarker for predicting poor clinical outcome in all of the most common subtypes of human breast cancer, including the more lethal triple negative subtype [35,36]. A loss of stromal Cav-1 predicts early tumor recurrence, lymph-node metastasis, tamoxifen-resistance, and poor survival. Overall, breast cancer patients with a loss of stromal Cav-1 show a 20% 5-year survival rate, compared to the 80% 5-year survival of patients with high stromal Cav-1 expression. In triple negative patients, the 5-year survival rate is 75.5% for high stromal Cav-1 versus 9.4% for absent stromal Cav-1 [37]. A loss of stromal Cav-1 also predicts progression to invasive disease in ductal carcinoma *in situ* patients, suggesting that a loss of Cav-1 regulates tumor progression [35]. Similarly, a loss of stromal Cav-1 is associated with advanced disease and metastasis, as well as a high Gleason score, in prostate cancer patients [38].

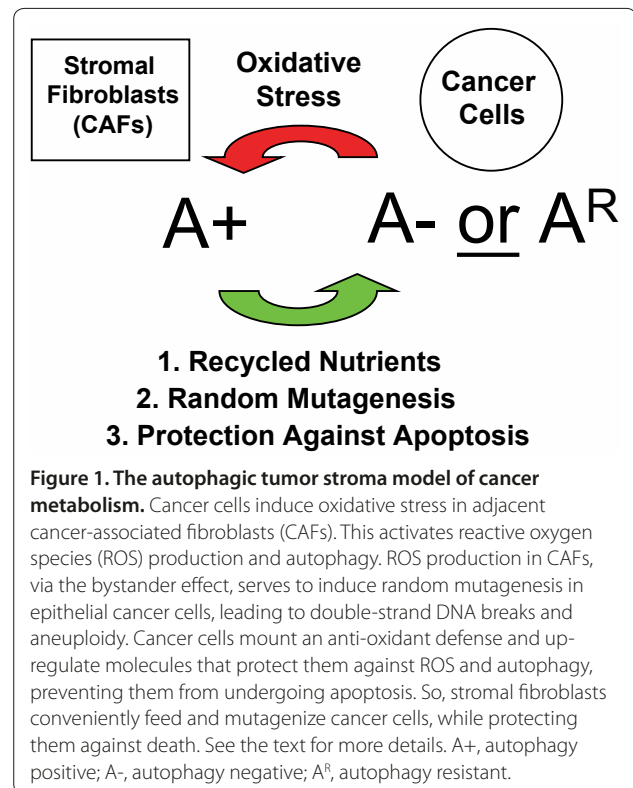
In order to mechanistically dissect how a loss of stromal Cav-1 induces a lethal microenvironment, we have employed Cav-1(-/-) null mice as a model system. Complementary studies were also performed with a co-culture model of normal human fibroblasts and MCF7 breast cancer cells.

Unbiased proteomic and transcriptional analysis has shown that Cav-1(-/-) null bone-marrow-derived stromal

cells display the up-regulation of both myo-fibroblast markers and glycolytic enzymes, clearly indicating that a loss of Cav-1 promotes myo-fibroblast conversion and induces aerobic glycolysis in stromal cells [39]. Thus, stromal cells lacking Cav-1 undergo aerobic glycolysis and secrete energy-rich metabolites (such as lactate and pyruvate) that directly feed cancer cells and fuel mitochondrial respiration of adjacent cancer cells. We have termed this novel hypothesis the 'reverse Warburg effect'. This novel idea has now also been independently confirmed by Pol and colleagues [40], who demonstrated that genetic ablation of Cav-1 in murine fibroblasts is indeed sufficient to functionally induce the onset of aerobic glycolysis via mitochondrial dysfunction [40]. Interestingly, immunohistochemistry on human breast cancers lacking stromal Cav-1 has demonstrated the over-expression of key glycolytic enzymes, such as PKM2 and LDH, in the fibroblastic tumor stroma [39].

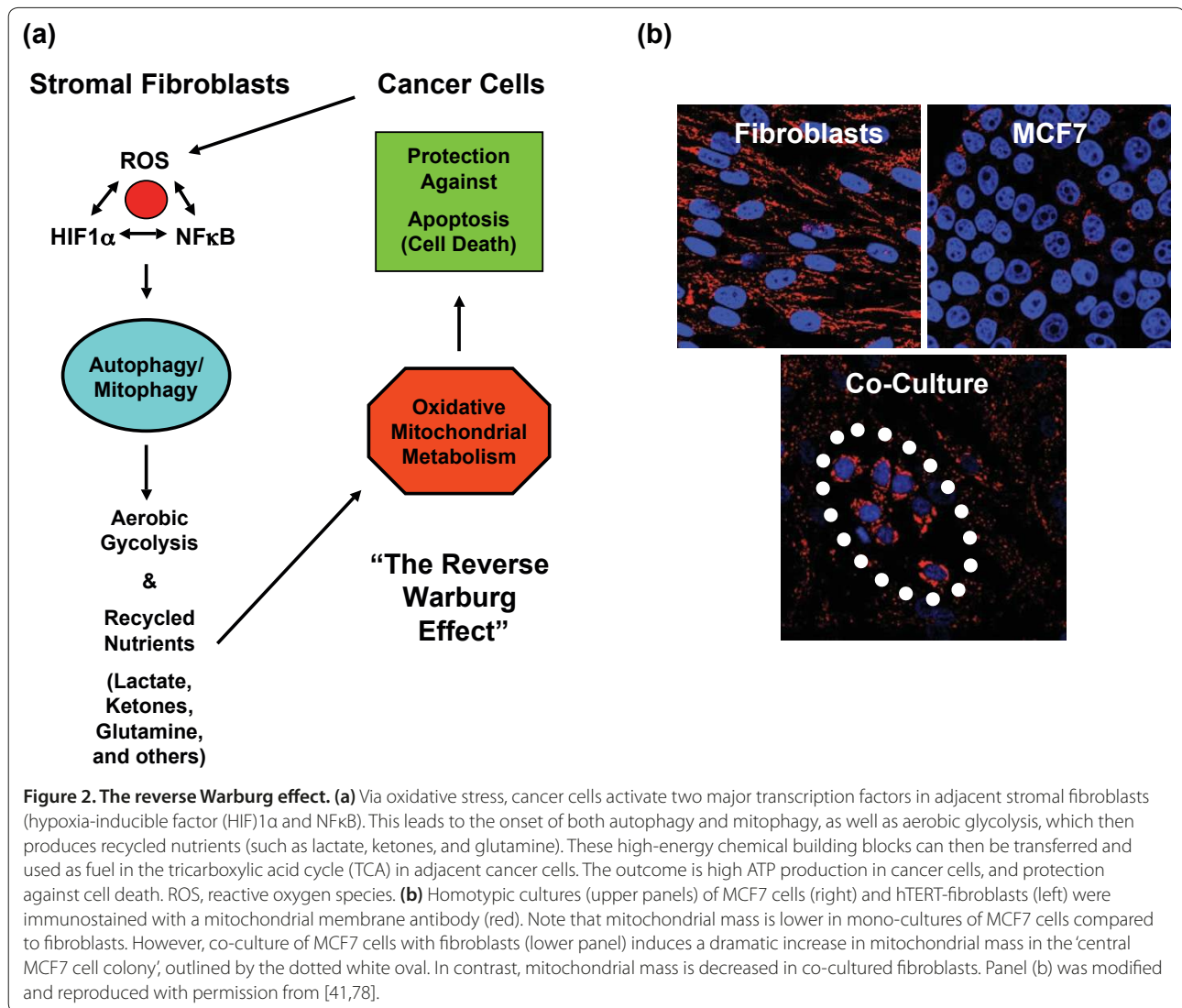
In further support of this hypothesis, co-culture with cancer cells promotes Cav-1 down-regulation in adjacent fibroblasts. Importantly, when cultured under homotypic conditions, MCF7 cancer cells have a very low mitochondrial mass (the conventional Warburg effect). However, co-culture with fibroblasts, which more closely mirrors the microenvironment of a naturally occurring tumor, promotes a very significant increase in mitochondrial mass in MCF7 cancer cells, suggesting that the Warburg effect might be an *in vitro* artifact [41]. Importantly, lactate administration to homotypic MCF7 cancer cell cultures significantly increases mitochondrial mass, suggesting that lactate administration phenocopies the presence of reactive fibroblasts [41], by promoting mitochondrial biogenesis. These new data indicate that cancer cells and CAFs develop a 'symbiotic' or 'parasitic' relationship, with the vectorial and unilateral transfer of energy from glycolytic stromal cells to oxidative cancer cells (Figures 1 and 2).

Clinically, high glucose uptake has been exploited to monitor tumor growth using position emission tomography (PET) scanning via radiolabeled 2-deoxy-glucose. However, we should acknowledge that PET avidity does not necessarily correlate with high aerobic glycolysis. For example, PET is not useful in clear cell renal carcinomas [42,43], which are the tumor 'prototype' in which the Warburg effect should sustain tumor formation. About half of clear cell renal carcinomas are due to mutations in the Von Hippel-Lindau (VHL) gene [44,45], leading to HIF1 $\alpha$  constitutive activation and forced induction of aerobic glycolysis [46-48]. Conversely, little attention is paid to understanding which cell-type or compartments within a tumor are most PET avid. In fact, the clinical use of PET is well established in Hodgkin's lymphomas [49], which are composed of less than 10% tumor cells, the rest being stromal and inflammatory cells [50]. Yet, Hodgkin's



lymphomas are very PET avid tumors, suggesting that 2-deoxy-glucose uptake may be associated with the tumor stroma. That the fibrotic component may be glucose avid is further supported by the notion that PET is clinically used to assess the therapeutic response in gastrointestinal stromal tumors (GIST), which are a subset of tumors of mesenchymal origin. Finally, pilot clinical studies have shown that PET is useful for detection of various forms of fibrosis, including pulmonary fibrosis [51].

The reverse Warburg effect can also be described as 'metabolic coupling' between supporting glycolytic stromal cells and oxidative tumor cells. Metabolic cooperativity between adjacent cell-compartments is observed in several normal physiological settings. During folliculogenesis and early embryogenesis, oocytes are surrounded by supporting cumulus granulosa (CG) cells. Oocytes lack some crucial metabolic functions, such as the ability to utilize glucose, to produce cholesterol and to transport certain amino acids. Thus, oocytes control CG cell metabolism, promoting their glycolysis, cholesterol synthesis and amino acid uptake [52,53]. Remarkably, both oocytes and zygotes can reach a two-cell stage only in the presence of pyruvate and oxaloacetate, but not using glucose [54,55]. However, upon co-culture with CG cells, oocytes or zygotes can cleave to a two-cell stage also in the presence of glucose, clearly indicating that CG cells metabolize glucose and supply the oocyte with the



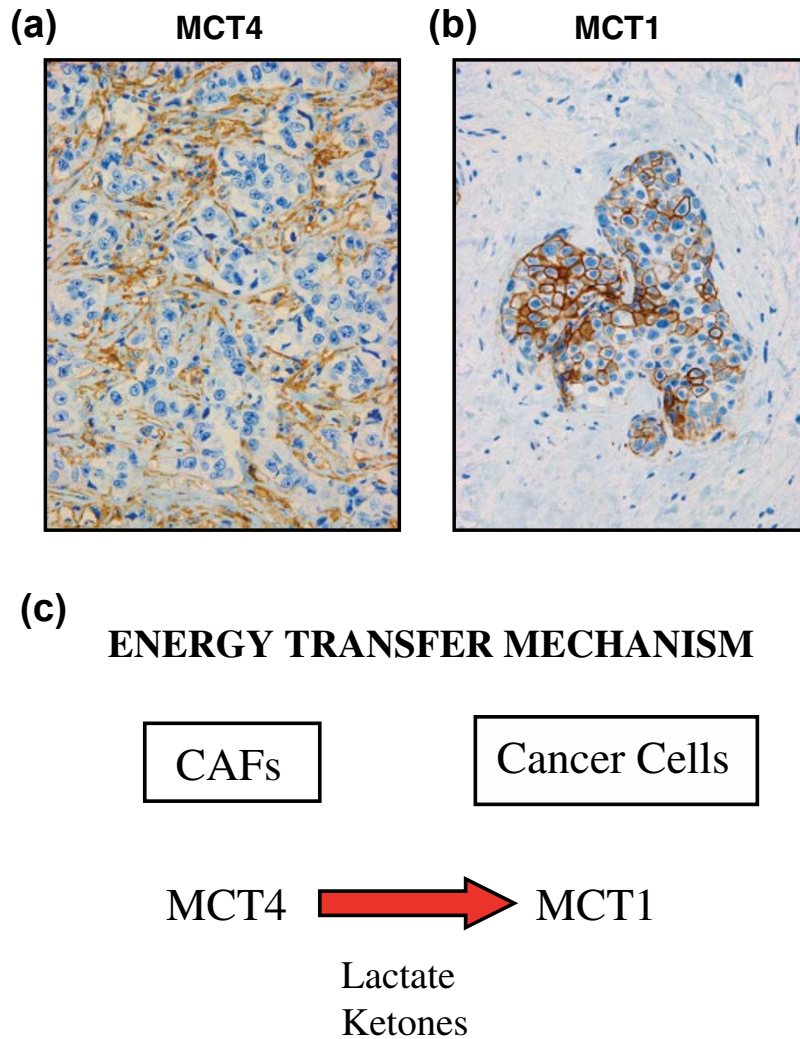
glycolytic product pyruvate to permit maturation [54]. In this regard, transcriptional profiling of CG cells and oocytes has revealed that key enzymes of the glycolytic pathway (aldolase A, enolase 1, LDHA, and PKM2) are very highly expressed in CG cells, but are undetectable in oocytes [56]. These results indicate that granulosa cells support the development and maturation of oocytes and early embryos by providing them with essential nutrients, such as oxidative phosphorylation substrates (pyruvate and lactate [57]), amino acids [58], and cholesterol [59].

Metabolic coupling is also normally observed in the brain between astrocytes and neurons, and this is known as ‘neuron-glia metabolic coupling’ [60,61]. In this regard, glycolytic astrocytes generate high levels of lactate to support mitochondrial oxidative phosphorylation in adjacent neurons. Consistent with this idea, LDH is preferentially expressed in astrocytes, and the mitochondrial enzyme pyruvate dehydrogenase is present

selectively within the neurons [62,63]. Also, in skeletal muscle it is well established that glycolytic fast-twitch muscle fibers produce lactate, which is then secreted via monocarboxylate transporter (MCT) 4. Lactate is then taken up by the slow-twitch fibers expressing MCT1 and utilized as a substrate for oxidative phosphorylation [64,65]. This form of metabolic coupling is known as the ‘skeletal muscle lactate shuttle’ [66].

As such, it is perhaps not surprising that tumors may have also developed a form of metabolic coupling, specifically involving tumor-stromal interactions. In further support of the existence of a ‘lactate shuttle’ in human tumors, we have now shown that CAFs express MCT4 (for lactate extrusion), while breast cancer cells express MCT1 (for lactate uptake) (Figure 3) [67]. Interestingly, MCT4 expression in CAFs is induced by oxidative stress, and MCT4 is a known HIF1α target gene (see Discussion below) [67].





**Figure 3. Evidence supporting a 'lactate shuttle' in human tumors: compartmentalized distribution of monocarboxylate transporter (MCT)1/4.** (a) MCT4 is expressed in the fibroblastic stromal compartment of human breast cancers. Note that MCT4 staining is absent from the tumor epithelial cells, but is present in the surrounding stroma. MCT4 staining outlines the cancer-associated fibroblasts that surround nests of epithelial cancer cells. (b) MCT1 is expressed in the epithelial compartment of human breast cancers. Note that MCT1 staining is present in the tumor epithelial cells, but is absent in the surrounding stroma. (c) The lactate shuttle: an energy transfer mechanism in normal tissue and human cancers. MCT4 functions primarily as a transporter that extrudes lactate from cells that are undergoing aerobic glycolysis and lack functional mitochondria. After lactate is extruded by MCT4 in cancer-associated fibroblasts (CAFs), lactate is then taken up by MCT1 in adjacent cancer cells. Similarly, ketones are transported by the same MCTs that handle lactate. Our studies suggest that metabolic coupling occurs between CAFs and adjacent tumor cells. Modified and reproduced with permission from [67].

### Oxidative stress: random mutagenesis and protection against cell death

Recent studies have dissected the mechanism(s) by which a loss of stromal Cav-1 leads to an aggressive breast cancer phenotype, and have shown that oxidative stress plays a central role. The role of oxidative stress in sustaining tumor growth is underscored by the observation that reactive oxygen species (ROS)-mediated myo-fibroblast conversion is sufficient to reduce tumor-free survival, and increases metastatic potential in a mammary tumor mouse model [68].

Gene expression profiling of Cav-1(-/-) null bone-marrow-derived stromal cells has shown the up-regulation of gene transcripts associated with ROS production, and over-expression of the transcriptional targets of HIF1 $\alpha$  and NF $\kappa$ B, suggesting that a loss of stromal Cav-1 induces oxidative stress, mimics hypoxia, and stimulates inflammation [41].

Co-cultures of normal human fibroblasts and MCF7 cells indicate that cancer cells use oxidative stress as a weapon to trigger the conversion of adjacent fibroblasts into myo-fibroblasts [69]. Cancer cell-induced oxidative

stress potently perturbs the behavior of adjacent fibroblasts, induces the lysosomal-mediated degradation of Cav-1, and promotes mitochondrial dysfunction, resulting in increased aerobic glycolysis [41]. In turn, these glycolytic fibroblasts support tumor cell mitochondrial respiration and growth by actively transferring high-energy nutrients (such as lactate and pyruvate) to cancer cells.

In support of the 'reverse Warburg effect', comparison of the tumor-promoting properties of two fibroblast lines (named CL3 and CL4) with either mitochondrial or glycolytic metabolism has revealed that aerobic glycolysis in CAFs greatly promotes tumor formation. CL3 fibroblasts show oxidative metabolism and increased mitochondrial mass, whereas CL4 fibroblasts display a shift towards aerobic glycolysis and increased lactate production [70]. Interestingly, in a xenograft model, CL4 fibroblasts enhance the growth of mammary tumors by approximately eight-fold compared to CL3 cells, without a detectable increase in angiogenesis. Consistent with this growth-promoting effect, CL4 fibroblasts also increase the mitochondrial mass of co-cultured breast cancer cells [71].

An oxidative-stress-rich micro-environment generates DNA damage in both cancer and stromal cells (Figure 1). We have shown that MCF7 cancer cells induce oxidative stress and promote DNA double-strand breaks in associated stromal cells, which are blocked by anti-oxidant treatments [41]. Similarly, after three-dimensional co-culture with prostate cancer cells, bone-derived stromal cells undergo stable cytogenetic modifications by a ROS-mediated mechanism [72].

Conversely, in an MCF7-fibroblast co-culture model, MCF7 cancer cells undergo aneuploidy and random mutagenesis [41], suggesting that CAFs facilitate the dynamic search for a more aggressive 'mutator phenotype' in cancer cells (Figure 1). Functionally, fibroblasts provide cancer cells with a six-fold protection against cell death [41] (Figure 2), via the up-regulation of the anti-apoptotic protein TIGAR [41]. Cav-1 knockdown fibroblasts provide even greater protection for cancer cells against apoptosis, clearly indicating that a loss of stromal Cav-1 in humans may greatly facilitate tumor growth by suppression of cancer cell death [41].

Thus, data supporting the 'reverse Warburg effect' indicate that cancer cells and fibroblasts are metabolically coupled and mutagenically co-evolving [41,73]. Cancer cells use oxidative stress to corrupt adjacent fibroblasts and to induce their metabolic re-programming [73]. In this way, fibroblasts secrete energy rich metabolites that facilitate cancer cell survival. In addition, fibroblasts promote mutagenesis of cancer cells, leading to a more aggressive 'aneuploid' phenotype [41,73]. Aneuploidy in cancer cells is known to be associated with poor clinical outcome.

### **Hypoxia, autophagy, and mitophagy in the tumor stroma**

Metabolomic profiling reveals that Cav-1(-/-) null mammary fat pads display a highly catabolic metabolism, with the increased release of several metabolites, such as amino acids, ribose and nucleotides, and a shift towards gluconeogenesis, as well as mitochondrial dysfunction [74]. These changes are consistent with increased autophagy, mitophagy and aerobic glycolysis, all processes that are induced by oxidative stress [74]. Autophagy or 'self-eating' is the process by which cells degrade their own cellular components to survive during starvation or to eliminate damaged organelles after oxidative stress. Mitophagy, or mitochondrial-autophagy, is particularly important to remove damaged ROS-generating mitochondria.

An autophagy/mitophagy program is also triggered by hypoxia [75,76]. Hypoxia is a common feature of solid tumors, and promotes cancer progression, invasion and metastasis [77]. Interestingly, via induction of autophagy, hypoxia is sufficient to induce a dramatic loss of Cav-1 in fibroblasts. The hypoxia-induced loss of Cav-1 can be inhibited by the autophagy inhibitor chloroquine, or by pharmacological inhibition of HIF1 $\alpha$  [69,78]. Conversely, small interfering RNA-mediated Cav-1 knock-down is sufficient to induce pseudo-hypoxia, with HIF1 $\alpha$  and NF $\kappa$ B activation, and to promote autophagy/mitophagy, as well as a loss of mitochondrial membrane potential in stromal cells [78]. These results indicate that a loss of stromal Cav-1 is a marker of hypoxia and oxidative stress. In a co-culture model, autophagy in cancer-associated fibroblasts was shown to promote tumor cell survival via the induction of the pro-autophagic HIF1 $\alpha$  and NF $\kappa$ B pathways in the tumor stromal microenvironment [78]. Finally, the mitophagy marker Bnip3L is selectively up-regulated in the stroma of human breast cancers lacking Cav-1, but is notably absent from the adjacent breast cancer epithelial cells [78].

Another study has shown that cell-specific induction of autophagy by HIF1 $\alpha$  activation in fibroblasts or MDA-MB-231 cells differentially affects tumor growth. In a xenograft model, HIF1 $\alpha$  activation in fibroblasts greatly enhances the tumorigenicity of co-injected MDA-MB-231 cells, whereas HIF1 $\alpha$  activation in MDA-MB-231 cancer cells suppresses tumor growth [79]. Importantly, in this experimental setting, the levels of tumor angiogenesis were unchanged. As HIF1 $\alpha$  triggers autophagy in both fibroblasts and cancer cells, these data demonstrate that the role of autophagy in driving tumor formation is cell-type specific, and that stromal autophagy, and not cancer cell autophagy, favors tumor growth.

Several studies have demonstrated that the over-expression of autophagic markers, such as ATG16L and cathepsin K and D, in the stroma and not in tumor cells

predicts poor prognosis [80-82]. Similarly, loss of autophagic markers, such as Beclin 1, in tumor cells correlates with poor clinical outcome, suggesting that activation of an autophagic program in tumor cells reduces tumor aggressiveness [83].

Metabolome profiling of several types of human cancer tissues versus corresponding normal tissues have consistently shown that cancer tissues are highly catabolic, with the significant accumulation of many amino acids and TCA cycle metabolites [84,85]. The levels of reduced glutathione were decreased in primary and metastatic prostate cancers compared to benign adjacent prostate tissue, suggesting that aggressive disease is associated with increased oxidative stress [86]. Also, these data show that the tumor microenvironment has increased oxidative-stress-induced autophagy and increased catabolism.

Taken together, all these findings suggest an integrated model whereby a loss of stromal Cav-1 induces autophagy/mitophagy in the tumor stroma, via oxidative stress. This creates a catabolic micro-environment with the local accumulation of chemical building blocks and recycled nutrients (such as amino acids and nucleotides), directly feeding cancer cells to sustain their survival and growth. We have termed this novel idea the 'autophagic tumor stroma model of cancer' [74]. This new paradigm may explain the 'autophagy paradox', which is based on the fact that both the systemic inhibition and systemic stimulation of autophagy prevent tumor formation [87]. We propose that vectorial energy transfer from the tumor stroma to cancer cells directly sustains tumor growth, and that interruption of such metabolic coupling will block tumor growth. Autophagy inhibitors (such as chloroquine) functionally block the catabolic transfer of metabolites from the stroma to the tumor, inducing cancer cell starvation and death [88]. Conversely, autophagy inducers (such as rapamycin) promote autophagy in tumor cells and induce cell death [89]. Thus, both inhibitors and inducers of autophagy will have a similar effect by severing the metabolic coupling of the stroma and tumor cells, resulting in tumor growth inhibition (cutting 'off' the fuel supply).

This model may also explain why enthusiasm for anti-angiogenic therapy has been dampened. In most cases, the clinical benefits are short term, and more importantly, new data suggest an unexpected link between anti-angiogenic treatments and metastasis. In pre-clinical models, anti-vascular endothelial growth factor (anti-VEGF) drugs (sunitinib and anti-VEGFR2 blocking antibodies) were shown to inhibit localized tumor formation, but potently induced relapse and metastasis [90-92]. Thus, by inducing hypoxia in the tumor microenvironment, anti-angiogenic drugs may create a more favorable metastatic niche [93]. Hypoxia-induced autophagy may play a role

by generating a catabolic micro-environment rich in chemical building blocks that can be directly used by cancer cells to sustain malignant transformation and metastatic progression.

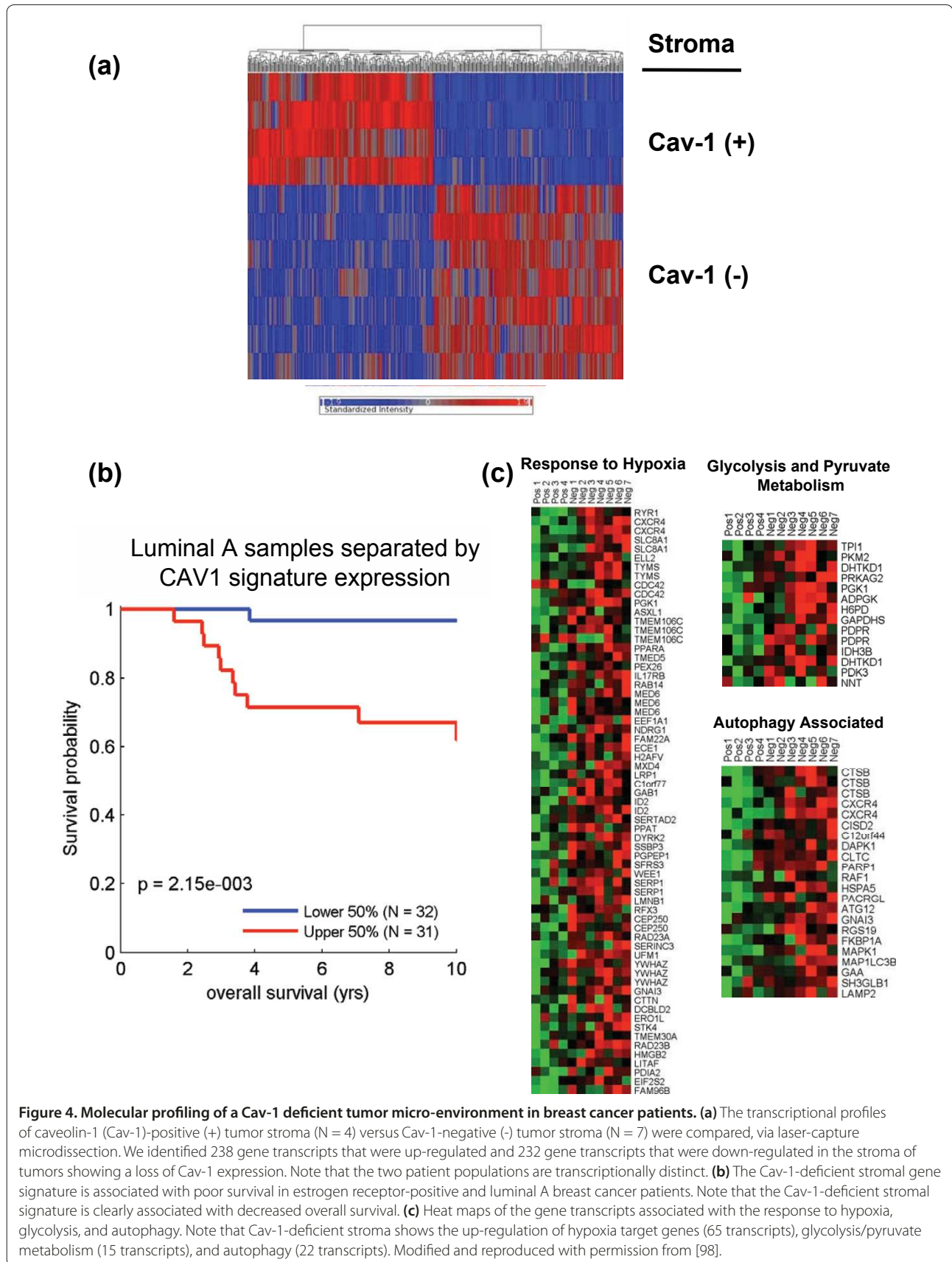
Finally, the autophagic tumor stroma model can also provide an explanation for systemic cachexia, which is progressive skeletal muscle and adipose tissue wasting, affecting up to 50% of all cancer patients and resulting in severe weight loss and shortened survival [94]. Cachexia is the result of increased energy consumption and higher metabolic rates [95]. Based on our data, we envision that cancer leads to a generalized catabolic state via an autophagic-mechanism that generates building blocks and starves the rest of the body. While the exact signaling pathways governing this phenomenon are not yet fully elucidated, it is clear that oxidative stress-induced autophagy functions as a driver of muscle wasting [96]. For example, skeletal muscles from tumor-bearing mice showed impaired Akt activation and a more than 50-fold induction of Bnip3, a well recognized marker of autophagy/mitophagy [97]. These findings also help explain why patients with metabolic syndrome and diabetes have an increased risk for the development of multiple epithelial cancers, due to their constitutive and systemic activation of the autophagic program, and the over-production of high-energy nutrients, such as lactate and ketones [74].

We have recently used laser-capture micro-dissection of the tumor stroma from human breast cancers to directly validate that a loss of stromal Cav-1 is transcriptionally associated with oxidative stress, hypoxia, autophagy, and mitochondrial dysfunction, via gene-set enrichment analysis (Figure 4) [98]. In addition, we see that oxidative stress in CAFs induces cytokine production via NF $\kappa$ B activation, directly linking inflammation with autophagy/catabolism in the tumor stroma [99]. So, cachexia may start locally as stromal autophagy, and then spread systemically via cytokine production and inflammation, which also drive autophagy [99].

### **Glutamine utilization, ammonia production, and autophagy in the tumor stroma**

In direct support that cancer cells use mitochondrial oxidative metabolism, many investigators have shown that cancer cells are 'addicted' to glutamine [24,100]. Glutamine is a non-essential amino acid that is metabolized to glutamate and enters the TCA cycle as alpha-ketoglutarate, resulting in high ATP generation via oxidative phosphorylation [2,22,101-103].

Recent studies also show that ammonia is a by-product of glutaminolysis [104-106]. In addition, ammonia can act as a diffusible inducer of autophagy [104-106]. Given these observations, glutamine addiction in cancer cells provides another mechanism for driving and/or





maintaining autophagy in the tumor micro-environment (Figure 5). In support of this idea, we have previously shown that a loss of Cav-1 in the stroma is sufficient to drive autophagy, resulting in increased glutamine production in the tumor micro-environment [74].

Thus, this concept defines a new vicious cycle in which autophagy in the tumor stroma transfers glutamine to cancer cells, and the by-product of this metabolism, ammonia, maintains autophagic glutamine production (Figure 5). This model fits well with the 'autophagic tumor stroma model of cancer metabolism', in which energy-rich recycled nutrients (lactate, ketones, and glutamine) fuel oxidative mitochondrial metabolism in cancer cells.

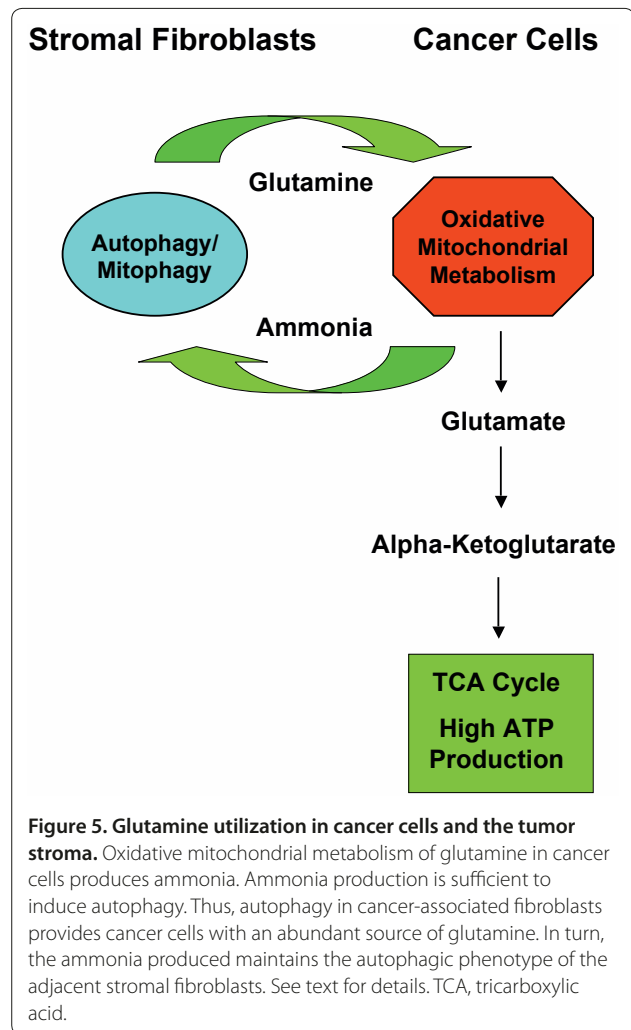
### Lessons from other paradigms: an infectious parasitic cancer cell that metastasizes and captures mitochondrial DNA from host cells

We have recently proposed that cancer cells behave like 'parasites', by inducing oxidative stress in normal host fibroblasts, resulting in the production of recycled nutrients via autophagy [41,69,78]. This is exactly the same mechanism by which infectious parasites (such as malaria) obtain nutrients and are propagated by inducing oxidative stress and autophagy in host cells [107-109]. In this regard, malaria is an 'intracellular' parasite, while cancer cells may be thought of as 'extracellular' parasites. This explains why chloroquine is both an effective anti-malarial drug and an effective anti-tumor agent, as it functions as an autophagy inhibitor [110], cutting off the 'fuel supply' in both disease states.

Are there any examples of cancer cells that act as infectious parasites? Surprisingly, the answer is yes [111]. There are four known types: canine transmissible venereal tumor (CTVT), which is a sexually transmitted disease that occurs in feral dogs [112] - it is transmitted through coitus, licking/biting, or sniffing; devil facial tumor disease, spread among Tasmanian devils via facial biting [113,114]; contagious reticulum cell sarcoma, which is transmitted between Syrian hamsters via mosquito bites [115,116]; and malignant fibrous histiocytoma, which was transmitted from a cancer patient to his surgeon [117] during an injury that occurred when the surgeon was operating.

These parasitic cancers are all transmitted by allografting, so the cancer cells literally 'metastasize' from one affected host to another 'naïve' host, in an infectious manner that does not involve a virus.

Experimentally, the most is known about CTVT. This disease is thought to have originated in ancient wolves or coyotes, and the tumor cells themselves act as the infectious agent. As such, these cancer cells are genetically distinct from their hosts, as determined by genomic sequence analysis of their nuclear DNA [118]. CTVT represents the oldest known cancer cell line that

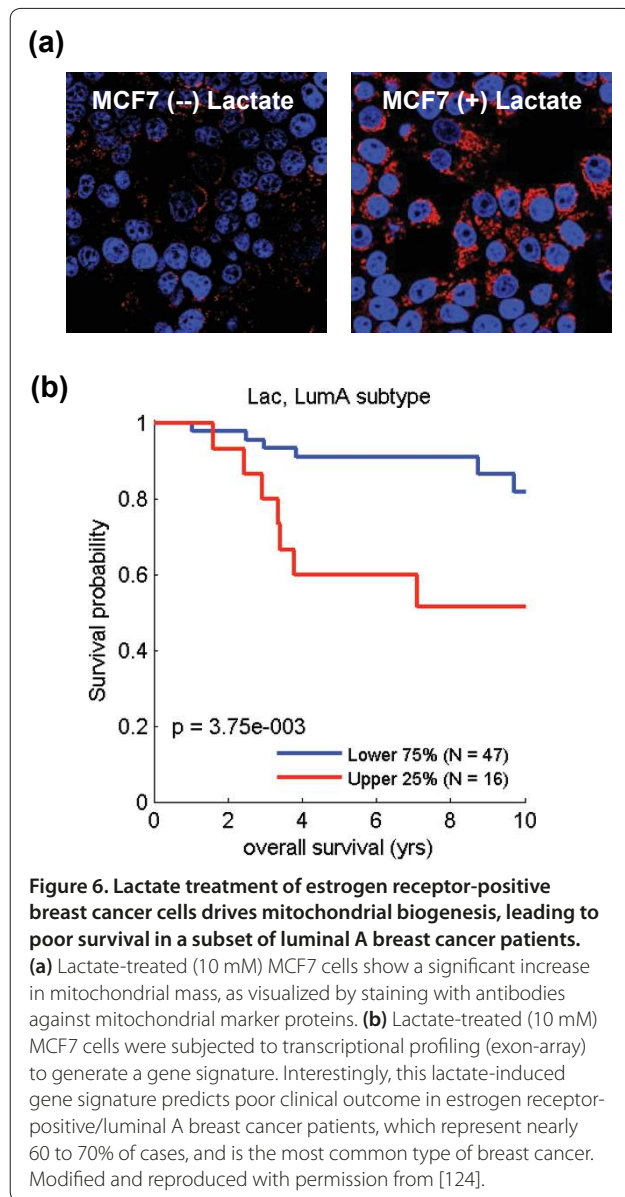


**Figure 5. Glutamine utilization in cancer cells and the tumor stroma.** Oxidative mitochondrial metabolism of glutamine in cancer cells produces ammonia. Ammonia production is sufficient to induce autophagy. Thus, autophagy in cancer-associated fibroblasts provides cancer cells with an abundant source of glutamine. In turn, the ammonia produced maintains the autophagic phenotype of the adjacent stromal fibroblasts. See text for details. TCA, tricarboxylic acid.

has been continuously propagated, most likely for more than 10,000 years [118,119].

Interestingly, it has recently been shown that CTVT tumor cells survive by periodically 'capturing' mitochondrial DNA from their hosts [119,120]. Thus, it has been suggested that these CVCT tumor cells have survived for more than 10,000 years by maintaining and renewing their capacity for oxidative mitochondrial metabolism by 'stealing' host cell mitochondrial DNA [119,120]. In accordance with this idea, CTVT is highly sensitive to adriamycin/doxorubicin therapy [121], a chemo-therapeutic agent that also functions as a mitochondrial poison.

Similarly, it has been independently shown that human cancer cells can 'steal' live mitochondria or mitochondrial DNA [122] from adjacent mesenchymal stem cells in culture, which then rescues aerobic glycolysis in these cancer cells [122]. This is known as mitochondrial transfer [122]. Interestingly, others have shown that metastatic breast cancer cells show the up-regulation of numerous mitochondrial proteins [123], specifically



associated with oxidative phosphorylation, as seen by unbiased proteomic analysis [123].

Thus, increased mitochondrial oxidative metabolism may be a key driver of tumor cell metastasis. In further support of this argument, treatment of MCF7 cancer cells with lactate is indeed sufficient to induce mitochondrial biogenesis in these cells [41,78] (Figure 6). To determine if these findings may be clinically relevant, a lactate-induced gene signature was recently generated using MCF7 cells [124]. This gene signature shows that lactate induces 'stemness' in cancer cells, and this lactate-induced gene signature predicts poor clinical outcome (including tumor recurrence and metastasis) in breast cancer patients [124] (Figure 6). These findings are consistent with experiments showing that intraperitoneal

injection of lactate in an MDA-MB-231 xenograft model results in an approximately ten-fold increase in lung metastasis [125].

#### Abbreviations

CAF, cancer associated fibroblast; Cav, caveolin; CTVT, canine transmissible venereal tumor; HIF, hypoxia-inducible factor; LDH, lactate dehydrogenase; MCT, monocarboxylate transporter; NF, nuclear factor; PET, position emission tomography; ROS, reactive oxygen species; TCA, tricarboxylic acid.

#### Competing interests

The authors declare that they have no competing interests.

#### Acknowledgements

MPL and his laboratory were supported by grants from the NIH/NCI (R01-CA-080250; R01-CA-098779; R01-CA-120876; R01-AR-055660), and the Susan G Komen Breast Cancer Foundation. FS was supported by grants from the WW Smith Charitable Trust, the Breast Cancer Alliance (BCA), and a Research Scholar Grant from the American Cancer Society (ACS). RGP was supported by grants from the NIH/NCI (R01-CA-70896, R01-CA-75503, R01-CA-86072, and R01-CA-107382) and the Dr Ralph and Marian C Falk Medical Research Trust. The Kimmel Cancer Center was supported by the NIH/NCI Cancer Center Core grant P30-CA-56036 (to RGP). Funds were also contributed by the Margaret Q Landenberger Research Foundation (to MPL). This project is funded, in part, under a grant with the Pennsylvania Department of Health (to MPL). The Department specifically disclaims responsibility for any analyses, interpretations or conclusions. This work was also supported, in part, by a Centre grant in Manchester from Breakthrough Breast Cancer in the UK (to AH) and an Advanced ERC Grant from the European Research Council. Dr MP Lisanti would especially like to thank Drs Craig B Thompson, Tak W Mak, Michel L Tremblay, and Robert S DiPaola (other participants of the ASBMB/AACR 2011 Meetings) for their feedback, thoughtful comments, and helpful suggestions. This review was recently presented as a lecture at the AACR 2011 Meeting in the Educational Session 'Autophagy and Cell Death' on 2 April 2011 (Orlando, FL). It was also presented as a lecture at the *Experimental Biology 2011-ASBMB Meeting* (Washington, DC) at the session on 'Metabolism and Cancer' on 13 April 2011.

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Published: 8 July 2011

#### References

1. Warburg O: On the origin of cancer cells. *Science* 1956, **123**:309-314.
2. Dang CV: PKM2 tyrosine phosphorylation and glutamine metabolism signal a different view of the Warburg effect. *Sci Signal* 2009, **2**:pe75.
3. Cairns RA, Harris IS, Mak TW: Regulation of cancer cell metabolism. *Nat Rev Cancer* 2011, **11**:85-95.
4. Deberardinis RJ, Sayed N, Ditsworth D, Thompson CB: Brick by brick: metabolism and tumor cell growth. *Curr Opin Genet Dev* 2008, **18**:54-61.
5. Guppy M, Leedman P, Zu X, Russell V: Contribution by different fuels and metabolic pathways to the total ATP turnover of proliferating MCF-7 breast cancer cells. *Biochem J* 2002, **364**:309-315.
6. Kallinowski F, Vaupel P, Runkel S, Berg G, Fortmeyer HP, Baessler KH, Wagner K, Mueller-Klieser W, Walenta S: Glucose uptake, lactate release, ketone body turnover, metabolic microclimate, and pH distributions in human breast cancer xenografts in nude rats. *Cancer Res* 1988, **48**:7264-7272.
7. Zu XL, Guppy M: Cancer metabolism: facts, fantasy, and fiction. *Biochem Biophys Res Commun* 2004, **313**:459-465.
8. Kallinowski F, Schlenger KH, Runkel S, Kloes M, Stohrer M, Okunieff P, Vaupel P: Blood flow, metabolism, cellular microenvironment, and growth rate of human tumor xenografts. *Cancer Res* 1989, **49**:3759-3764.
9. Gstraunthaler G, Seppi T, Pfaller W: Impact of culture conditions, culture

- media volumes, and glucose content on metabolic properties of renal epithelial cell cultures. Are renal cells in tissue culture hypoxic? *Cell Physiol Biochem* 1999, **9**:150-172.
10. Koukourakis MI, Giatromanolaki A, Harris AL, Sivridis E: **Comparison of metabolic pathways between cancer cells and stromal cells in colorectal carcinomas: a metabolic survival role for tumor-associated stroma.** *Cancer Res* 2006, **66**:632-637.
  11. Yan H, Parsons DW, Jin G, McLendon R, Rasheed BA, Yuan W, Kos I, Batnisch-Haberle I, Jones S, Riggins GJ, Friedman H, Friedman A, Reardon D, Herndon J, Kinzler KW, Velculescu VE, Vogelstein B, Bigner DD: **IDH1 and IDH2 mutations in gliomas.** *N Engl J Med* 2009, **360**:765-773.
  12. Fogal V, Richardson AD, Karmali PP, Scheffler IE, Smith JW, Ruoslahti E: **Mitochondrial p32 protein is a critical regulator of tumor metabolism via maintenance of oxidative phosphorylation.** *Mol Cell Biol* 2010, **30**:1303-1318.
  13. Sheen-Chen SM, Huang CC, Tang RP, Chou FF, Eng HL: **Prognostic value of signal transducers and activators of transcription 3 in breast cancer.** *Cancer Epidemiol Biomarkers Prev* 2008, **17**:2286-2290.
  14. Wegrzyn J, Potla R, Chwae YJ, Sepuri NB, Zhang Q, Koeck T, Derecka M, Szczepanek K, Szelag M, Gornicka A, Moh A, Moghaddas S, Chen Q, Bobbili S, Cichy J, Dulak J, Baker DP, Wolfman A, Stuehr D, Hassan MQ, Fu XY, Avadhani N, Drake JJ, Fawcett P, Lesnfsky EJ, Larner AC: **Function of mitochondrial Stat3 in cellular respiration.** *Science* 2009, **323**:793-797.
  15. Reich NC: **STAT3 revs up the powerhouse.** *Sci Signal* 2009, **2**:pe61.
  16. Gough DJ, Corlett A, Schlessinger K, Wegrzyn J, Larner AC, Levy DE: **Mitochondrial STAT3 supports Ras-dependent oncogenic transformation.** *Science* 2009, **324**:1713-1716.
  17. Weinberg F, Hamanaka R, Wheaton WW, Weinberg S, Joseph J, Lopez M, Kalyanaraman B, Mutlu GM, Budinger GR, Chandel NS: **Mitochondrial metabolism and ROS generation are essential for Kras-mediated tumorigenicity.** *Proc Natl Acad Sci U S A* 2010, **107**:8788-8793.
  18. Berridge MV, Tan AS: **Effects of mitochondrial gene deletion on tumorigenicity of metastatic melanoma: reassessing the Warburg effect.** *Rejuvenation Res* 2010, **13**:139-141.
  19. Yu M, Shi Y, Wei X, Yang Y, Zhou Y, Hao X, Zhang N, Niu R: **Depletion of mitochondrial DNA by ethidium bromide treatment inhibits the proliferation and tumorigenesis of T47D human breast cancer cells.** *Toxicol Lett* 2007, **170**:83-93.
  20. Sakamaki T, Casimiro MC, Ju X, Quong AA, Katiyar S, Liu M, Jiao X, Li A, Zhang X, Lu Y, Wang C, Byers S, Nicholson R, Link T, Shemluck M, Yang J, Fricke ST, Novikoff PM, Papanikolaou A, Arnold A, Albanese C, Pestell R: **Cyclin D1 determines mitochondrial function in vivo.** *Mol Cell Biol* 2006, **26**:5449-5469.
  21. Bostrom P, Soderstrom M, Palokangas T, Vahlberg T, Collan Y, Carpen O, Hirsimaki P: **Analysis of cyclins A, B1, D1 and E in breast cancer in relation to tumour grade and other prognostic factors.** *BMC Res Notes* 2009, **2**:140.
  22. Gao P, Tchernyshyov I, Chang TC, Lee YS, Kita K, Ochi T, Zeller KI, De Marzo AM, Van Eyk JE, Mendell JT, Dang CV: **c-Myc suppression of miR-23a/b enhances mitochondrial glutaminase expression and glutamine metabolism.** *Nature* 2009, **458**:762-765.
  23. Wise DR, DeBerardinis RJ, Mancuso A, Sayed N, Zhang XY, Pfeiffer HK, Nissim I, Daikhin E, Yudkoff M, McMahon SB, Thompson CB: **Myc regulates a transcriptional program that stimulates mitochondrial glutaminolysis and leads to glutamine addiction.** *Proc Natl Acad Sci U S A* 2008, **105**:18782-18787.
  24. Wang JB, Erickson JW, Fuji R, Ramachandran S, Gao P, Dinavahi R, Wilson KF, Ambrosio AL, Dias SM, Dang CV, Cerione RA: **Targeting mitochondrial glutaminase activity inhibits oncogenic transformation.** *Cancer Cell* 2010, **18**:207-219.
  25. Kalluri R, Zeisberg M: **Fibroblasts in cancer.** *Nat Rev Cancer* 2006, **6**:392-401.
  26. Ronnov-Jessen L, Petersen OW, Bissell MJ: **Cellular changes involved in conversion of normal to malignant breast: importance of the stromal reaction.** *Physiol Rev* 1996, **76**:69-125.
  27. Shekhar MP, Pauley R, Heppner G: **Host microenvironment in breast cancer development: extracellular matrix-stromal cell contribution to neoplastic phenotype of epithelial cells in the breast.** *Breast Cancer Res* 2003, **5**:130-135.
  28. Orimo A, Gupta PB, Sgroi DC, Arenzana-Seisdedos F, Delaunay T, Naeem R, Carey VJ, Richardson AL, Weinberg RA: **Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion.** *Cell* 2005, **121**:335-348.
  29. Karnoub AE, Dash AB, Vo AP, Sullivan A, Brooks MW, Bell GW, Richardson AL, Polyak K, Tubo R, Weinberg RA: **Mesenchymal stem cells within tumour stroma promote breast cancer metastasis.** *Nature* 2007, **449**:557-563.
  30. Wu M, Jung L, Cooper AB, Fleet C, Chen L, Breault L, Clark K, Cai Z, Vincent S, Bottega S, Shen Q, Richardson A, Bosenburg M, Naber SP, DePinho RA, Kuperwasser C, Robinson MO: **Dissecting genetic requirements of human breast tumorigenesis in a tissue transgenic model of human breast cancer in mice.** *Proc Natl Acad Sci U S A* 2009, **106**:7022-7027.
  31. Howell A, Landberg G, Bergh J: **Breast tumour stroma is a prognostic indicator and target for therapy.** *Breast Cancer Res* 2009, **11** Suppl 3:S16.
  32. Finak G, Bertos N, Pepin F, Sadekova S, Souleimanova M, Zhao H, Chen H, Omeroglu G, Meterissian S, Omeroglu A, Hallett M, Park M: **Stromal gene expression predicts clinical outcome in breast cancer.** *Nat Med* 2008, **14**:518-527.
  33. Ma XJ, Dahiya S, Richardson E, Erlander M, Sgroi DC: **Gene expression profiling of the tumor microenvironment during breast cancer progression.** *Breast Cancer Res* 2009, **11**:R7.
  34. Bianchini G, Qi Y, Alvarez RH, Iwamoto T, Coutant C, Ibrahim NK, Valero V, Cristofanilli M, Green MC, Radvanyi L, Hatzis C, Hortobagyi GN, Andre F, Gianni L, Symmans WF, Pusztai L: **Molecular anatomy of breast cancer stroma and its prognostic value in estrogen receptor-positive and -negative cancers.** *J Clin Oncol* 2010, **28**:4316-4323.
  35. Witkiewicz AK, Casimiro MC, Dasgupta A, Mercier I, Wang C, Bonuccelli G, Jasmin JF, Frank PG, Pestell RG, Kleer CG, Sotgia F, Lisanti MP: **Towards a new "stromal-based" classification system for human breast cancer prognosis and therapy.** *Cell Cycle* 2009, **8**:1654-1658.
  36. Sloan EK, Ciocca DR, Pouliot N, Natoli A, Restall C, Henderson MA, Fanelli MA, Cuellar-Carrion FD, Gago FE, Anderson RL: **Stromal cell expression of caveolin-1 predicts outcome in breast cancer.** *Am J Pathol* 2009, **174**:2035-2043.
  37. Witkiewicz AK, Dasgupta A, Sammons S, Er O, Potoczek MB, Guiles F, Sotgia F, Brody JR, Mitchell EP, Lisanti MP: **Loss of stromal caveolin-1 expression predicts poor clinical outcome in triple negative and basal-like breast cancers.** *Cancer Biol Ther* 2010, **10**:135-143.
  38. Di Vizio D, Morello M, Sotgia F, Pestell RG, Freeman MR, Lisanti MP: **An absence of stromal caveolin-1 is associated with advanced prostate cancer, metastatic disease and epithelial Akt activation.** *Cell Cycle* 2009, **8**:2420-2424.
  39. Pavlides S, Whitaker-Menezes D, Castello-Cros R, Flomenberg N, Witkiewicz AK, Frank PG, Casimiro MC, Wang C, Fortina P, Addya S, Pestell RG, Martinez-Outschoorn UE, Sotgia F, Lisanti MP: **The reverse Warburg effect: aerobic glycolysis in cancer associated fibroblasts and the tumor stroma.** *Cell Cycle* 2009, **8**:3984-4001.
  40. Bosch M, Mari M, Herms A, Fernández A, Fajardo A, Kassar A, Giral A, Colell A, Balmora D, Barbero E, González-Moreno E, Matias N, Tebar F, Balsinde J, Camps M, Enrich C, Gross SP, García-Ruiz C, Pérez-Navarro E, Fernández-Checa JC, Pol A: **Caveolin-1 deficiency causes cholesterol-dependent mitochondrial dysfunction and apoptotic susceptibility.** *Curr Biol* 2011, **21**:681-686.
  41. 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: **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* 2010, **9**:3256-3276.
  42. Mueller-Lisse UG, Mueller-Lisse UL: **Imaging of advanced renal cell carcinoma.** *World J Urol* 2010, **28**:253-261.
  43. Avril N, Dambha F, Murray I, Shamash J, Powles T, Sahdev A: **The clinical advances of fluorine-2-D-deoxyglucose-positron emission tomography/computed tomography in urological cancers.** *Int J Urol* 2010, **17**:501-511.
  44. Gnarr JR, Tory K, Weng Y, Schmidt L, Wei MH, Li H, Latif F, Liu S, Chen F, Duh FM, et al: **Mutations of the VHL tumour suppressor gene in renal carcinoma.** *Nat Genet* 1994, **7**:85-90.
  45. Cowey CL, Rathmell WK: **VHL gene mutations in renal cell carcinoma: role as a biomarker of disease outcome and drug efficacy.** *Curr Oncol Rep* 2009, **11**:94-101.
  46. Tanimoto K, Makino Y, Pereira T, Poellinger L: **Mechanism of regulation of the hypoxia-inducible factor-1 alpha by the von Hippel-Lindau tumor suppressor protein.** *EMBO J* 2000, **19**:4298-4309.
  47. Kamura T, Sato S, Iwai K, Czyzyk-Krzeska M, Conaway RC, Conaway JW: **Activation of HIF1alpha ubiquitination by a reconstituted von**



- Hippel-Lindau (VHL) tumor suppressor complex. *Proc Natl Acad Sci U S A* 2000, **97**:10430-10435.
48. Ohh M, Park CW, Ivan M, Hoffman MA, Kim TY, Huang LE, Pavletich N, Chau V, Kaelin WG: **Ubiquitination of hypoxia-inducible factor requires direct binding to the beta-domain of the von Hippel-Lindau protein.** *Nat Cell Biol* 2000, **2**:423-427.
49. Weiler-Sagie M, Bushelov O, Epelbaum R, Dann EJ, Haim N, Avivi I, Ben-Barak A, Ben-Arie Y, Bar-Shalom R, Israel O: **(18)F-FDG avidity in lymphoma readdressed: a study of 766 patients.** *J Nucl Med* 2010, **51**:25-30.
50. Aldinucci D, Gloghini A, Pinto A, De Filippi R, Carbone A: **The classical Hodgkin's lymphoma microenvironment and its role in promoting tumour growth and immune escape.** *J Pathol* 2010, **221**:248-263.
51. Groves AM, Win T, Screaton NJ, Berovic M, Endozo R, Booth H, Kayani I, Menezes LJ, Dickson JC, Ell PJ: **Idiopathic pulmonary fibrosis and diffuse parenchymal lung disease: implications from initial experience with 18F-FDG PET/CT.** *J Nucl Med* 2009, **50**:538-545.
52. Su YQ, Sugiura K, Eppig JJ: **Mouse oocyte control of granulosa cell development and function: paracrine regulation of cumulus cell metabolism.** *Semin Reprod Med* 2009, **27**:32-42.
53. Sugiura K, Su YQ, Diaz FJ, Pangas SA, Sharma S, Wigglesworth K, O'Brien MJ, Matzuk MM, Shimasaki S, Eppig JJ: **Oocyte-derived BMP15 and FGFs cooperate to promote glycolysis in cumulus cells.** *Development* 2007, **134**:2593-2603.
54. Biggers JD, Whittingham DG, Donahue RP: **The pattern of energy metabolism in the mouse oocyte and zygote.** *Proc Natl Acad Sci U S A* 1967, **58**:560-567.
55. Brinster RL: **Studies on the development of mouse embryos in vitro. II. The effect of energy source.** *J Exp Zool* 1965, **158**:59-68.
56. Sugiura K, Pendola FL, Eppig JJ: **Oocyte control of metabolic cooperativity between oocytes and companion granulosa cells: energy metabolism.** *Dev Biol* 2005, **279**:20-30.
57. Brinster RL: **Oxidation of pyruvate and glucose by oocytes of the mouse and rhesus monkey.** *J Reprod Fertil* 1971, **24**:187-191.
58. Colonna R, Mangia F: **Mechanisms of amino acid uptake in cumulus-enclosed mouse oocytes.** *Biol Reprod* 1983, **28**:797-803.
59. Su YQ, Sugiura K, Wigglesworth K, O'Brien MJ, Affourtit JP, Pangas SA, Matzuk MM, Eppig JJ: **Oocyte regulation of metabolic cooperativity between mouse cumulus cells and oocytes: BMP15 and GDF9 control cholesterol biosynthesis in cumulus cells.** *Development* 2008, **135**:111-121.
60. Tsacopoulos M, Magistretti PJ: **Metabolic coupling between glia and neurons.** *J Neurosci* 1996, **16**:877-885.
61. Magistretti PJ, Pellerin L: **Cellular mechanisms of brain energy metabolism. Relevance to functional brain imaging and to neurodegenerative disorders.** *Ann N Y Acad Sci* 1996, **777**:380-387.
62. Bittar PG, Charnay Y, Pellerin L, Bouras C, Magistretti PJ: **Selective distribution of lactate dehydrogenase isoenzymes in neurons and astrocytes of human brain.** *J Cereb Blood Flow Metab* 1996, **16**:1079-1089.
63. Laughton JD, Bittar P, Charnay Y, Pellerin L, Kovari E, Magistretti PJ, Bouras C: **Metabolic compartmentalization in the human cortex and hippocampus: evidence for a cell- and region-specific localization of lactate dehydrogenase 5 and pyruvate dehydrogenase.** *BMC Neurosci* 2007, **8**:35.
64. Connett RJ, Gayeski TE, Honig CR: **Lactate accumulation in fully aerobic, working, dog gracilis muscle.** *Am J Physiol* 1984, **246**:H120-128.
65. McCullagh KJ, Poole RC, Halestrap AP, Tipton KF, O'Brien M, Bonen A: **Chronic electrical stimulation increases MCT1 and lactate uptake in red and white skeletal muscle.** *Am J Physiol* 1997, **273**:E239-246.
66. Brooks GA: **The lactate shuttle during exercise and recovery.** *Med Sci Sports Exerc* 1986, **18**:360-368.
67. Whitaker-Menezes D, Martinez-Outschoorn UE, Lin Z, Ertel A, Flomenberg N, Witkiewicz AK, Birbe RC, Howell A, Pavlides S, Gandara R, Pestell RG, Sotgia F, Philp NJ, Lisanti MP: **Evidence for a stromal-epithelial 'lactate shuttle' in human tumors: MCT4 is a marker of oxidative stress in cancer-associated fibroblasts.** *Cell Cycle* 2011, **10**:1772-1783.
68. Toullec A, Gerald D, Despouy G, Bourachot B, Cardon M, Lefort S, Richardson M, Rigault G, Parrini MC, Lucchesi C, Bellanger D, Stern MH, Dubois T, Sastre-Garau X, Delattre O, Vincent-Salomon A, Mehta-Grigoriou F: **Oxidative stress promotes myofibroblast differentiation and tumour spreading.** *EMBO Mol Med* 2010, **2**:211-230.
69. 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: **Tumor cells induce the cancer associated fibroblast phenotype via caveolin-1 degradation: Implications for breast cancer and DCIS therapy with autophagy inhibitors.** *Cell Cycle* 2010, **9**:2423-2433.
70. Ramanathan A, Wang C, Schreiber SL: **Perturbational profiling of a cell-line model of tumorigenesis by using metabolic measurements.** *Proc Natl Acad Sci U S A* 2005, **102**:5992-5997.
71. Migneco G, Whitaker-Menezes D, Chiavarina B, Castello-Cros R, Pavlides S, Pestell RG, Fatatis A, Flomenberg N, Tsirigos A, Howell A, Martinez-Outschoorn UE, Sotgia F, Lisanti MP: **Glycolytic cancer associated fibroblasts promote breast cancer tumor growth, without a measurable increase in angiogenesis: Evidence for stromal-epithelial metabolic coupling.** *Cell Cycle* 2010, **9**:2412-2422.
72. Sung SY, Hsieh CL, Law A, Zhou HE, Pathak S, Multani AS, Lim S, Coleman IM, Wu LC, Figg WD, Dahut WL, Nelson P, Lee JK, Amin MB, Lyles R, Johnstone PA, Marshall FF, Chung LW: **Coevolution of prostate cancer and bone stroma in three-dimensional coculture: implications for cancer growth and metastasis.** *Cancer Res* 2008, **68**:9996-10003.
73. Lisanti MP, Martinez-Outschoorn UE, Chiavarina B, Pavlides S, Whitaker-Menezes D, Tsirigos A, Witkiewicz A, Lin Z, Balliet R, Howell A, Sotgia F: **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* 2011, **10**:537-542.
74. Pavlides S, Tsirigos A, Migneco G, Whitaker-Menezes D, Chiavarina B, Flomenberg N, Frank PG, Casimiro MC, Wang C, Pestell RG, Martinez-Outschoorn UE, Howell A, Sotgia F, Lisanti MP: **The autophagic tumor stroma model of cancer: Role of oxidative stress and ketone production in fueling tumor cell metabolism.** *Cell Cycle* 2010, **9**:3485-3505.
75. Azad MB, Gibson SB: **Role of BNIP3 in proliferation and hypoxia-induced autophagy: implications for personalized cancer therapies.** *Ann N Y Acad Sci* 2010, **1210**:8-16.
76. Kume S, Uzu T, Horiike K, Chin-Kanasaki M, Isshiki K, Araki S, Sugimoto T, Haneda M, Kashiwagi A, Koya D: **Calorie restriction enhances cell adaptation to hypoxia through Sirt1-dependent mitochondrial autophagy in mouse aged kidney.** *J Clin Invest* 2010, **120**:1043-1055.
77. Pennacchietti S, Michieli P, Galluzzo M, Mazzone M, Giordano S, Comoglio PM: **Hypoxia promotes invasive growth by transcriptional activation of the met protooncogene.** *Cancer Cell* 2003, **3**:347-361.
78. Martinez-Outschoorn UE, Trimmer C, Lin Z, Whitaker-Menezes D, Chiavarina B, Zhou J, Wang C, Pavlides S, Martinez-Cantarin MP, Capozza F, Witkiewicz AK, Flomenberg N, Howell A, Pestell RG, Caro J, Lisanti MP, Sotgia F: **Autophagy in cancer associated fibroblasts promotes tumor cell survival: Role of hypoxia, HIF1 induction and NFKappaB activation in the tumor stromal microenvironment.** *Cell Cycle* 2010, **9**:3515-3533.
79. Chiavarina B, Whitaker-Menezes D, Migneco G, Martinez-Outschoorn UE, Pavlides S, Howell A, Tanowitz HB, Casimiro MC, Wang C, Pestell RG, Grieshaber P, Caro J, Sotgia F, Lisanti MP: **HIF1-alpha functions as a tumor promoter in cancer associated fibroblasts, and as a tumor suppressor in breast cancer cells: Autophagy drives compartment-specific oncogenesis.** *Cell Cycle* 2010, **9**:3534-3551.
80. Nomura H, Uzawa K, Yamano Y, Fushimi K, Ishigami T, Kouzu Y, Koike H, Siiba M, Bukawa H, Yokoe H, Kubosawa H, Tanzawa H: **Overexpression and altered subcellular localization of autophagy-related 16-like 1 in human oral squamous-cell carcinoma: correlation with lymphovascular invasion and lymph-node metastasis.** *Hum Pathol* 2009, **40**:83-91.
81. Nadji M, Fresno M, Nassiri M, Conner G, Herrero A, Morales AR: **Cathepsin D in host stromal cells, but not in tumor cells, is associated with aggressive behavior in node-negative breast cancer.** *Hum Pathol* 1996, **27**:890-895.
82. Kleer CG, Bloushtain-Qimron N, Chen YH, Carrasco D, Hu M, Yao J, Kraeft SK, Collins LC, Sabel MS, Argani P, Gelman R, Schnitt SJ, Krop IE, Polyak K: **Epithelial and stromal cathepsin K and CXCL14 expression in breast tumor progression.** *Clin Cancer Res* 2008, **14**:5357-5367.
83. Levine B, Kroemer G: **Autophagy in the pathogenesis of disease.** *Cell* 2008, **132**:27-42.
84. Denkert C, Budczies J, Kind T, Weichert W, Tablack P, Sehoul J, Niesporek S, Konsgen D, Dietel M, Fiehn O: **Mass spectrometry-based metabolic profiling reveals different metabolite patterns in invasive ovarian carcinomas and ovarian borderline tumors.** *Cancer Res* 2006, **66**:10795-10804.
85. Hirayama A, Kami K, Sugimoto M, Sugawara M, Toki N, Onozuka H, Kinoshita T, Saito N, Ochiai A, Tomita M, Esumi H, Soga T: **Quantitative metabolome profiling of colon and stomach cancer microenvironment by capillary**



- electrophoresis time-of-flight mass spectrometry. *Cancer Res* 2009, **69**:4918-4925.
86. Sreekumar A, Poisson LM, Rajendiran TM, Khan AP, Cao Q, Yu J, Laxman B, Mehra R, Lonigro RJ, Li Y, Nyati MK, Ahsan A, Kalyana-Sundaram S, Han B, Cao X, Byun J, Omenn GS, Ghosh D, Pennathur S, Alexander DC, Berger A, Shuster JR, Wei JT, Varambally S, Beecher C, Chinnaiyan AM: **Metabolomic profiles delineate potential role for sarcosine in prostate cancer progression.** *Nature* 2009, **457**:910-914.
87. Eisenberg-Lerner A, Kimchi A: **The paradox of autophagy and its implication in cancer etiology and therapy.** *Apoptosis* 2009, **14**:376-391.
88. Maclean KH, Dorsey FC, Cleveland JL, Kastan MB: **Targeting lysosomal degradation induces p53-dependent cell death and prevents cancer in mouse models of lymphomagenesis.** *J Clin Invest* 2008, **118**:79-88.
89. Sparks CA, Guertin DA: **Targeting mTOR: prospects for mTOR complex 2 inhibitors in cancer therapy.** *Oncogene* 2010, **29**:3733-3744.
90. Loges S, Mazzone M, Hohensinner P, Carmeliet P: **Silencing or fueling metastasis with VEGF inhibitors: antiangiogenesis revisited.** *Cancer Cell* 2009, **15**:167-170.
91. Paez-Ribes M, Allen E, Hudock J, Takeda T, Okuyama H, Vinals F, Inoue M, Bergers G, Hanahan D, Casanovas O: **Antiangiogenic therapy elicits malignant progression of tumors to increased local invasion and distant metastasis.** *Cancer Cell* 2009, **15**:220-231.
92. Ebos JM, Lee CR, Cruz-Munoz W, Bjarnason GA, Christensen JG, Kerbel RS: **Accelerated metastasis after short-term treatment with a potent inhibitor of tumor angiogenesis.** *Cancer Cell* 2009, **15**:232-239.
93. Blagosklonny MV: **Antiangiogenic therapy and tumor progression.** *Cancer Cell* 2004, **5**:13-17.
94. Tisdale MJ: **Cancer cachexia.** *Curr Opin Gastroenterol* 2010, **26**:146-151.
95. Holmes S: **A difficult clinical problem: diagnosis, impact and clinical management of cachexia in palliative care.** *Int J Palliat Nurs* 2009, **15**:320, 322-326.
96. McClung JM, Judge AR, Powers SK, Yan Z: **p38 MAPK links oxidative stress to autophagy-related gene expression in cachectic muscle wasting.** *Am J Physiol Cell Physiol* 2010, **298**:C542-549.
97. Asp ML, Tian M, Wendel AA, Belury MA: **Evidence for the contribution of insulin resistance to the development of cachexia in tumor-bearing mice.** *Int J Cancer* 2010, **126**:756-763.
98. Witkiewicz AK, Kline J, Queenan M, Brody JR, Tsirigos A, Pavlides S, Sotgia F, Lisanti MP: **Molecular profiling of a lethal tumor microenvironment, as defined by stromal caveolin-1 status in breast cancers.** *Cell Cycle* 2011, **10**:1794-1809.
99. Martinez-Outschoorn UE, Whitaker-Menezes D, Lin Z, Flomenberg N, Howell A, Pestell RG, Lisanti MP, Sotgia F: **Cytokine production and inflammation drive autophagy in the tumor microenvironment: Role of stromal caveolin-1 as a key regulator.** *Cell Cycle* 2011, **10**:1784-1793.
100. Dang CV: **Glutaminolysis: supplying carbon or nitrogen or both for cancer cells?** *Cell Cycle* 2010, **9**:3884-3886.
101. Wise DR, Thompson CB: **Glutamine addiction: a new therapeutic target in cancer.** *Trends Biochem Sci* 2010, **35**:427-433.
102. Dang CV, Le A, Gao P: **MYC-induced cancer cell energy metabolism and therapeutic opportunities.** *Clin Cancer Res* 2009, **15**:6479-6483.
103. Kaadige MR, Looper RE, Kamalanaadhan S, Ayer DE: **Glutamine-dependent anapleurosis dictates glucose uptake and cell growth by regulating MondoA transcriptional activity.** *Proc Natl Acad Sci U S A* 2009, **106**:14878-14883.
104. Eng CH, Abraham RT: **Glutaminolysis yields a metabolic by-product that stimulates autophagy.** *Autophagy* 2010, **6**:968-970.
105. Eng CH, Yu K, Lucas J, White E, Abraham RT: **Ammonia derived from glutaminolysis is a diffusible regulator of autophagy.** *Sci Signal* 2010, **3**:ra31.
106. Marino G, Kroemer G: **Ammonia: a diffusible factor released by proliferating cells that induces autophagy.** *Sci Signal* 2010, **3**:pe19.
107. Dey S, Guha M, Alam A, Goyal M, Bindu S, Pal C, Maity P, Mitra K, Bandyopadhyay U: **Malarial infection develops mitochondrial pathology and mitochondrial oxidative stress to promote hepatocyte apoptosis.** *Free Radic Biol Med* 2009, **46**:271-281.
108. Postma NS, Mommers EC, Eling WM, Zuidema J: **Oxidative stress in malaria; implications for prevention and therapy.** *Pharm World Sci* 1996, **18**:121-129.
109. Siddiqi NJ, Alhomida AS: **Status of hepatic oxidative stress and antioxidant defense systems during chloroquine treatment of *Plasmodium yoelii* nigeriensis infected mice.** *In Vivo* 1999, **13**:547-550.
110. Solomon VR, Lee H: **Chloroquine and its analogs: a new promise of an old drug for effective and safe cancer therapies.** *Eur J Pharmacol* 2009, **625**:220-233.
111. Murchison EP: **Clonally transmissible cancers in dogs and Tasmanian devils.** *Oncogene* 2008, **27** Suppl 2:S19-30.
112. Thomas R, Rebbeck C, Leroi AM, Burt A, Breen M: **Extensive conservation of genomic imbalances in canine transmissible venereal tumors (CTVT) detected by microarray-based CGH analysis.** *Chromosome Res* 2009, **17**:927-934.
113. McCallum H, Jones M, Hawkins C, Hamede R, Lachish S, Sinn DL, Beeton N, Lazenby B: **Transmission dynamics of Tasmanian devil facial tumor disease may lead to disease-induced extinction.** *Ecology* 2009, **90**:3379-3392.
114. Kreiss A, Tovar C, Obendorf DL, Dun K, Woods GM: **A murine xenograft model for a transmissible cancer in Tasmanian devils.** *Vet Pathol* 2011, **48**:475-481.
115. Copper HL, Mackay CM, Banfield WG: **Chromosome studies of a contagious reticulum cell sarcoma of the Syrian hamster.** *J Natl Cancer Inst* 1964, **33**:691-706.
116. Banfield WG, Woke PA, Mackay CM, Cooper HL: **Mosquito transmission of a reticulum cell sarcoma of hamsters.** *Science* 1965, **148**:1239-1240.
117. Gartner HV, Seidl C, Luckenbach C, Schumm G, Seifried E, Ritter H, Bultmann B: **Genetic analysis of a sarcoma accidentally transplanted from a patient to a surgeon.** *N Engl J Med* 1996, **335**:1494-1496.
118. Rebbeck CA, Thomas R, Breen M, Leroi AM, Burt A: **Origins and evolution of a transmissible cancer.** *Evolution* 2009, **63**:2340-2349.
119. Rebbeck CA, Leroi AM, Burt A: **Mitochondrial capture by a transmissible cancer.** *Science* 2011, **331**:303.
120. Villanueva T: **Metabolism: the mitochondria that wag the dog.** *Nat Rev Cancer* 2011, **11**:155.
121. Stettner N, Brenner O, Eilam R, Harmelin A: **Pegylated liposomal doxorubicin as a chemotherapeutic agent for treatment of canine transmissible venereal tumor in murine models.** *J Vet Med Sci* 2005, **67**:1133-1139.
122. Spees JL, Olson SD, Whitney MJ, Prockop DJ: **Mitochondrial transfer between cells can rescue aerobic respiration.** *Proc Natl Acad Sci U S A* 2006, **103**:1283-1288.
123. Chen EI, Hewel J, Krueger JS, Tiraby C, Weber MR, Kralli A, Becker K, Yates JR 3rd, Felding-Habermann B: **Adaptation of energy metabolism in breast cancer brain metastases.** *Cancer Res* 2007, **67**:1472-1486.
124. Martinez-Outschoorn UE, Prisco M, Ertel A, Tsirigos A, Lin Z, Pavlides S, Wang C, Flomenberg N, Knudsen ES, Howell A, Pestell RG, Sotgia F, Lisanti MP: **Ketones and lactate increase cancer cell "stemness," driving recurrence, metastasis, and poor clinical outcome in breast cancer: Achieving personalized medicine via Metabolo-Genomics.** *Cell Cycle* 2011, **10**:1271-1286.
125. 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: **Ketones and lactate "fuel" tumor growth and metastasis: Evidence that epithelial cancer cells use oxidative mitochondrial metabolism.** *Cell Cycle* 2010, **9**:3506-3514.

doi:10.1186/bcr2892  
Cite this article as: Sotgia F, et al.: Understanding the Warburg effect and the prognostic value of stromal caveolin-1 as a marker of a lethal tumor microenvironment. *Breast Cancer Research* 2011, **13**:213.