Review Article

Warburg effect and its role in tumourigenesis

Running title: Warburg and cancer

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Abstract

Glucose is a crucial molecule in energy production and produces different end products in non-tumourigenic- and tumourigenic tissue metabolism. Tumourigenic cells oxidise glucose by fermentation and generate lactate and adenosine triphosphate (ATP) even in the presence of oxygen (Warburg effect). The Na^+/H^+-antiporter is upregulated in tumourigenic cells resulting in release of lactate- and H^+ ions into the extracellular space. Accumulation of lactate- and proton ions in the extracellular space results in an acidic environment that promotes invasion and metastasis. Otto Warburg reported that tumourigenic cells have defective mitochondria that produce less energy. However, decades later it became evident that these mitochondria have adapted with alterations in mitochondrial content, structure, function and activity. Mitochondrial biogenesis and mitophagy regulate the formation of new mitochondria and degradation of defective mitochondria in order to combat accumulation of mutagenic mitochondrial deoxyribonucleic acid. Tumourigenic cells also produce increase reactive oxygen species (ROS) resulting from upregulated glycolysis leading to pathogenesis including cancer. Moderate ROS levels exert proliferative- and prosurvival signaling, while high ROS quantities induce cell death. Understanding the crosstalk between aberrant metabolism, redox regulation, mitochondrial adaptions and pH regulation provides scientific- and medical communities with new opportunities to explore cancer therapies.

Keywords: Cancer, Acidity, Mitochondria, ROS, Biogenesis, Mitophagy

Introduction

Glucose is an essential molecule for cells since it serves as the main substrate for producing energy (adenosine triphosphate (ATP)) in eukaryotic cell (Hardy 2015; Liberti 2016). Glucose metabolism is differentially regulated in tumourigenic- and non-tumourigenic cells. Non-tumourigenic cells metabolise glucose by means of glycolysis resulting in the production of pyruvate which is taken through to the tricarboxylic acid cycle (TCA) and subsequently to oxidative phosphorylation (OXPHOS) where ATP is produced (DeBerardinis 2008; Jin 2016).

In most tumourigenic cells, glucose undergoes glycolysis followed by the rapid conversion of pyruvate into lactate even in the presence of oxygen (DeBerardinis 2008; Lu 2015; Jin 2016). Furthermore, tumourigenic cells possess upregulated rates of glycolysis and produce ATP although glycolysis is accompanied with increased yields of lactic acid and H^+ ions (Stubbs 2000). Lactate and H^+ ions exit the cell into the extracellular space via the monocarboxylate carrier in order to maintain a neutral intracellular pH (Figure 1). An influx of H^+ ions into the extracellular space causes a decrease in pH (~6.8) which then promotes local invasion and metastasis and thereby favours tumourigenesis (Stubbs 2000).

This review focuses on the contribution of the Warburg effect to tumourigenesis and addresses signaling of aberrant tumourigenic metabolism regarding the microenvironment, mitochondria and mitophagy.

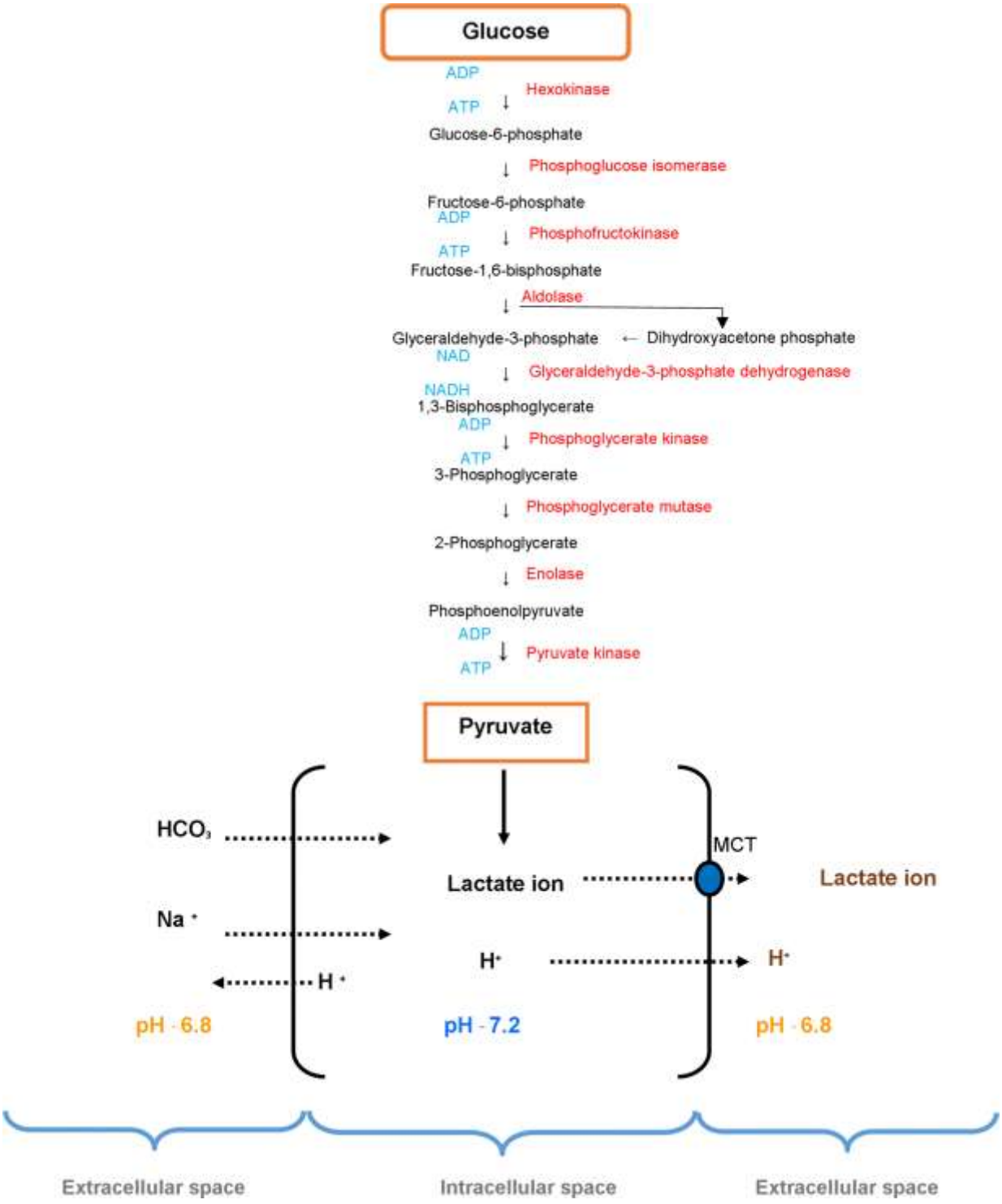

Figure 1. H^+ ions and lactate are transported to the extracellular space in order to maintain a neutral intracellular environment. When lactic acid is formed through glycolysis, lactate and H^+ ions leave the cell through the monocarboxylate carrier. The Na^+/H^+ antiporter is activated in tumour cells, allowing influx of Na^+ ions and outflow of H^+ ions. Entry of HCO_3^- ions into the cell serves as a buffer (diagram created by M.T Lebelo in Microsoft Publisher 2013).

Glucose metabolism in cancer and the Warburg effect

In the year 1924, Otto Warburg reported that tumourigenic cells consume more glucose when compared to non-tumourigenic cells (Lu 2015; Liberti 2016) (Table 1 and Figure 2). The latter was demonstrated by comparing tumourigenic liver tissue with non-tumourigenic liver tissue where significantly less oxygen was used by the tumourigenic liver tissue when compared to the non-tumourigenic cells. However, glucose metabolism was ten-fold higher than expected and lactic acid quantities were significantly elevated in the tumourigenic cells (Warburg 1927). Subsequent research demonstrated that tumour viability is dependent on the availability and supply of glucose and oxygen (Warburg 1927). Inhibition of glycolysis resulted in cell death in colon tumourigenic cells (HCT116) and lymphoma cells (Raji) (Cazzaniga 2015). However, different tumour types have differential respiratory capability and glycolytic activity depending on growth factor signaling (Liberti 2016). Differential glucose metabolism was confirmed in lung tumours from non-small cell lung cancer (NSCLC) mouse models and xenograft tumours derived from human cancer cell lines (A549- and H1975) when compared to non-tumourigenic tissue (Davidson 2016). Tumourigenic cells enhance glucose uptake by means of upregulating glycolysis and promoting the activation of oncogenes including myelocytomatosis viral oncogene homolog (MYC), the Ras-related oncogenes and 5' adenosine monophosphate-activated protein kinase (AMPK), hypoxia-inducible factor 1 (HIF-1), phosphoinositide 3-kinase (PI3K), protein kinase B (AKT) and the suppression of tumour suppressor protein p53 (TP53) (Sui 2004; DeBerardinis 2008; Jeon 2012; Jeon 2015; Liberti and Locasale 2016).

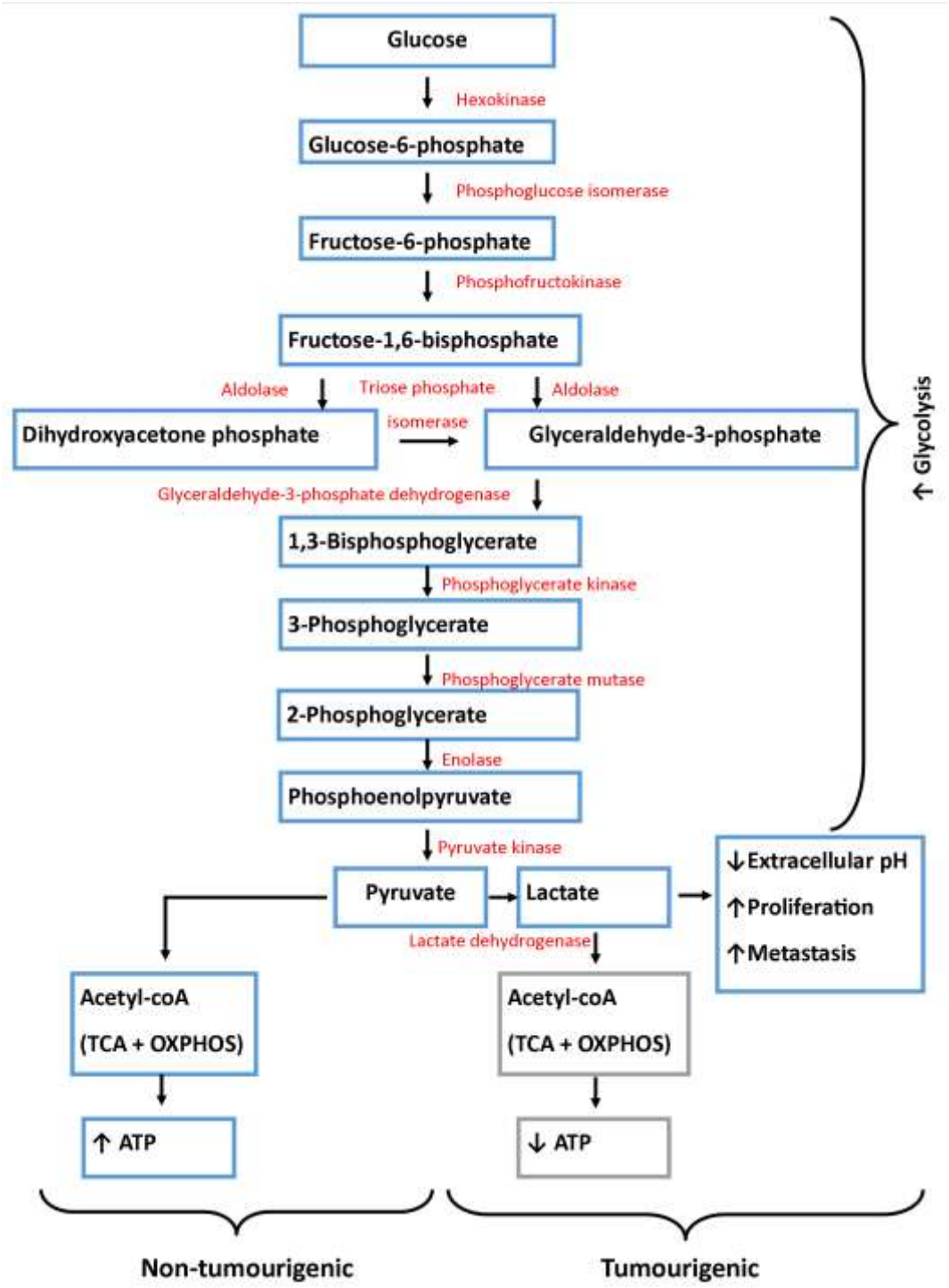

Figure 2. In non-tumourigenic cells, glycolysis takes place resulting in production of pyruvate which is converted to acetyl-coA that goes through to the TCA cycle and oxidative phosphorylation yielding maximum ATP. In tumourigenic cells, there is an increased rate of glycolysis to compensate for the low ATP yield through glycolysis. Furthermore, in tumourigenic- or transformed cells, pyruvate is converted to lactate which results in an acidic microenvironment, metastasis and ultimately, tumour cell growth. Only a minimal amount of pyruvate goes through to oxidative phosphorylation (diagram created by M.T Lebelo in Microsoft Publisher 2013).

Table 1: Differential glucose metabolism in tumourigenic- and non-tumourigenic cells [14-19].

Metabolism in tumourigenic cells	**Metabolism in non-tumourigenic cells**
Glycolysis	Glycolysis
Pyruvate→lactate	Pyruvate→acetyl co-enzyme
Minimal number of pyruvate molecules that undergo OXPHOS	Maximum number of pyruvate molecules undergo OXPHOS
Yield 2 ATP from 1 glucose molecule through glycolysis	Yield 36 ATP from 1 glucose molecule through OXPHOS +2 ATP through glycolysis
Upregulates glucose uptake for increased ATP production	Excess ATP has an inhibitory effect on glycolysis
Upregulation of glucose transporters	Glucose transporters not upregulated

Glucose uptake is significantly higher in tumourigenic cells due to increased protein production and membrane translocation of facilitative glucose transporters (GLUTs) (Gonzalez-Mendendez 2018). The GLUT protein family consists of 13 members of and 3 subclasses including Class I GLUTs (GLUTs1-4) which are glucose transporters, Class II GLUTs (GLUTs 5, 7, 9, 10) which are fructose transporters and Class III (GLUTs 6, 8, 10, 12 and the myo-inositol transporter HMIT1) which are structurally atypical when compared to the other GLUTs and poorly defined until date. Studies have indicated that several GLUTs are upregulated in tumourigenic tissue compared to non-tumourigenic tissue including GLUT1, GLUT3, GLUT4 and GLUT6 (Gu 2018). Moreover, GLUT expression and localization is augmented in response to nutrient deprivation. In non-tumourigenic cells, subsequent to nutrient deprivation GLUT1 is internalised and degraded by lysosomes resulting in decreased metabolism prior to induction of cell death (Gonzalez-Mendendez

2018). However, tumourigenic cells overexpress GLUT1 after nutrient deprivation or hypoxia in order to maintain glucose metabolism and become resistant to apoptosis (Gonzalez-Mendendez 2018). GLUT1 is overexpressed in several types of cancer including hepatic, pancreatic, breast, oesophageal, brain, renal, lung, cutaneous, colorectal, endometrial, ovarian and cervical. Thus, several studies have proposed that GLUTs and specifically, GLUT1 be evaluated as a target for anticancer therapy. Antibodies targeting GLUT1 decreased proliferation by 50% and 75% in a non-small cell lung cancer cell line and breast cancer cell lines, respectively. In addition, antibodies targeting GLUT1 augmented the antiproliferative effects of cisplatin, paclitaxel, and gefitinib (Wang 2019).

GLUT1 expression is also increased by HIFs, transcriptional factors, activated during hypoxia conditions resulting from upregulated glycolysis and encourages the expression of cell survival genes including vascular endothelial growth factor (VEGF) (Aquino-Gálvez 2016; Barteczek 2016). Tumourigenic cells further achieve sufficient energy via glycolysis by upregulating glucose transporters (GLUT) which increases glucose uptake into the cell (Cazzaniga 2015). GLUT1 overexpression is correlated with poor prognosis in cancer diagnosis and is prevalently overexpressed in several types of cancer including brain, breast, colon, kidney, lung, ovary and prostate (Bellance 2009). Identified GLUT inhibitors include resveratrol (RSV), naringenin, phorectin, WZB117, thiazolidinedione and STF-31 (Chiche 2009; Estrella 2013). Exposure to these compounds in cancer cell lines reduced proliferation and increased sensitivity to chemotherapy Chiche 2009. Thiazolidinedione decreased viability of prostate metastatic (LNCaP) cells and WZB117 inhibited proliferation in the human adenocarcinoma alveolar basal epithelial (A549) cell line (Siebeneicher 2016). It was reported that RSV reduced glucose uptake interrupted intracellular GLUT1 transport to the

plasma membrane and induced apoptosis in ovarian cancer cells (Wieman 2007; Gwak 2015).

Other reported glucose transporters in eukaryotic cells include sodium-glucose sympoters (SGLTs) and a new class of sugar transporters (SWEETs) which includes uniporter, SLC50. Preliminary studies have reported that SLC50A1 can potentially be used as a serum-based diagnostic and prognostic biomarker in breast cancer where data indicated that serum SLC50A1 levels discriminate between females with breast cancer and healthy females with a sensitivity of 75% and a specificity of 100%. Furthermore, a positive correlation revealed unfavourable 3 year outcomes in patients presenting with high-grade breast cancer (Szablewski 2013).

AMPK, a cellular energy sensor, is involved in tumour growth and survival in severe microenvironments (i.e. altered metabolism) (Jeon 2012; Jeon 2015). AMPK is activated by means of phosphorylation when 5' adenosine monophosphate (AMP) quantities are increased and ATP quantities are decreased, which is suggestive of energy deficiency (Liu 2019). AMPK consists of α (63 kD), β (30 kD), and γ (37–63 kD) subunits. The α subunit is responsible for regulation of cellular energy by means of by either downregulating anabolic processes or upregulating catabolic pathways in order to replenish cellular ATP. This is partly accomplished by activating Akt by means of PI3K which are pro-survival pathways resulting in downstream activation of several targets and processes including regulation of GLUT1 localisation and stimulation of hexokinase activity within glycolysis. Furthermore, GLUT1 expression is significantly decreased by means of the liver kinase B1

(LKB1)/AMPK/PI3K/AKT pathways after introduction of miRNA-451 in Glioma cells by targeting calcium binding protein 39 (CAB39) (Guo 2016).

An important transcription factor in glucose uptake is Yin Yang 1 (YY1), believed to play a role in cell proliferation and development (Sui 2004; Kasim 2017). Upregulation of YY1 in various kinds of cancers including breast, prostate and colon, this indicates the important role YY1 plays in cancer cell metabolism (Wu 2013). Studies have reported on YY1's role in tumourigenesis through the regulation of p53. Loss of YY1 resulted in a massive increase in p53 whereas overexpression of YY1 resulted in loss of p53 (Sui 2004; Wu 2013). Recent studies demonstrated the role of YY1 in tumourigenesis through a direct link to GLUT3 which has a high affinity for glucose (figure 3) (Wang 2018). Inhibition of YY1/GLUT3 resulted in an antiproliferative effect in colon carcinoma cells which illustrates the importance of YY1/GLUT axis in cancer cell proliferation (Wang 2018).

Since tumourigenic cells are dependent on upregulated aerobic glycolysis for energy production when compared to non-tumourigenic cells, glycolytic inhibitors provide clinicians with novel opportunities to target cancer cells (Siebeneicher 2016). Thus, targeting the increased level of aerobic glycolysis (Warburg effect) in anticancer treatment is a promising research field for future studies. A recent study found that wagonin exerted significant antitumor activity *in vitro* in colon cancer cell lines (HCT116, HT-29 and p53null HCT116) by means of inhibiting glycolysis and blocking tumor-suppressor function of p53. Wagonin also reduced tumor growth in nude mice inoculated with ovarian cancer cells (A2780) and colon cancer cells (HT-29) (Zhao 2018).

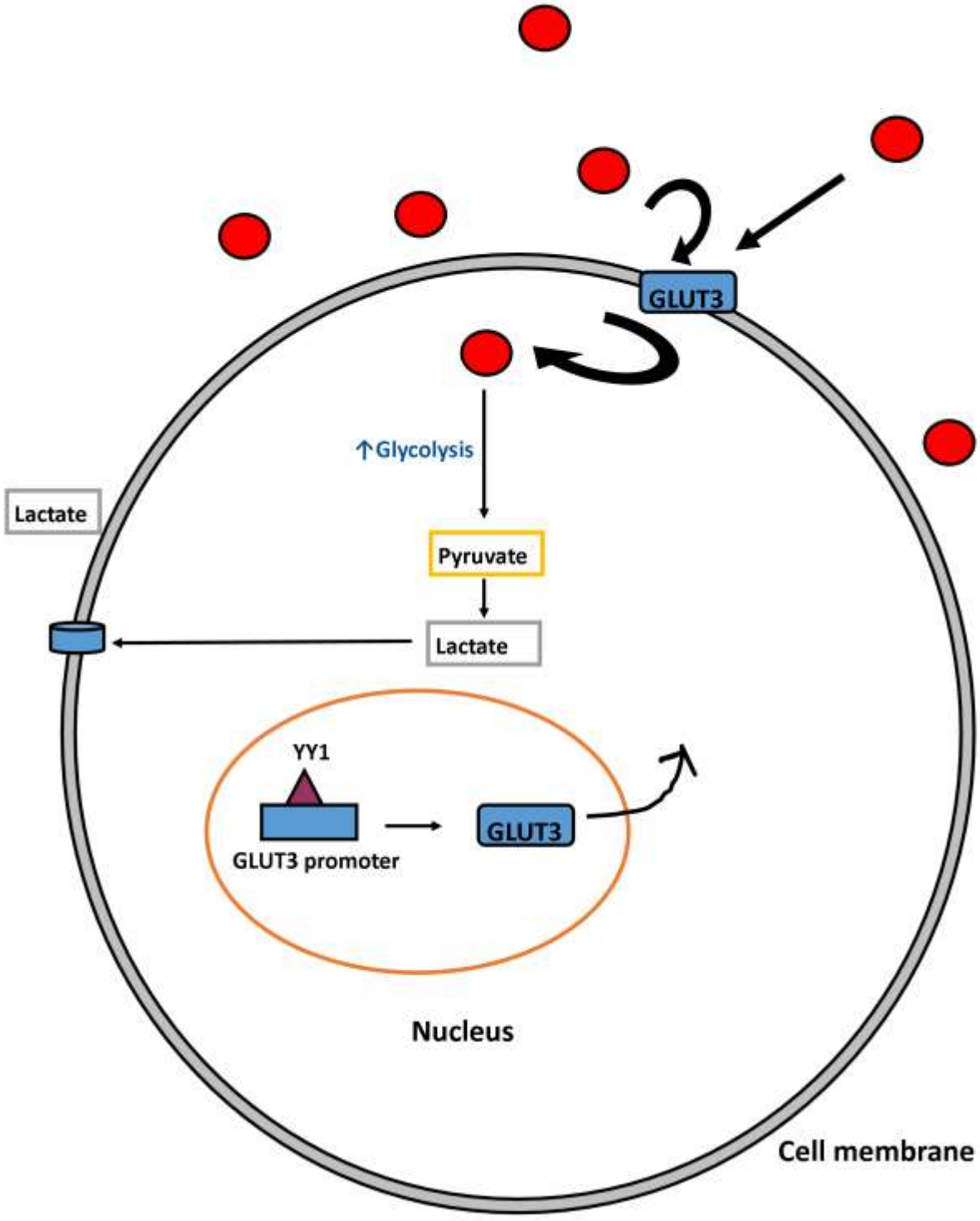

Figure 3. YY1 activates GLUT3 by binding to its promotor which results in increased oxygen consumption due to GLUT3's high affinity for glucose. The lactate is transported out into the extracellular space contributing to the acidic microenvironment. This drives aerobic glycolysis in some tumourigenic cells.

The glycolytic pathway comprises of a series of 10 reactions. Enzymes involved in the glycolytic pathway are all potential targets for inhibitors used in experimental anticancer therapy (Siebeneicher 2016). Hexokinase, the first regulatory enzyme in glycolysis is responsible for the phosphorylation of glucose to glucose-6-phopshate. Hexokinase exists in various isoforms including I, II, III and IV. Hexokinase II is frequently found overexpressed in several types of cancer including pancreatic- and liver cancer (Gwak 2015).

Researchers have demonstrated that glycolysis can be regulated at a post-transcriptional level by means of microRNA (miRNA), small non-coding ribonucleic acid, that modulate gene expression by binding to the 3'-untranslated regions (3'UTRs) of target mRNA. miR-145 inhibits glycolysis by means of targeting hexokinase II resulting in less energy production (Derouet 2018; Zhang 2018). Tumour suppressor activity of miR-145 has been observed in a variety of cancer types including breast, lung, colon and stomach pertaining to antiproliferative activity, inhibition of cell survival, induction of apoptosis, cell cycle arrest and inhibition of migration (Zhang 2018). miR-145 directly targets 3'UTRs of Hepatitis B virus X-interacting protein (HBXIP) that is responsible for cancer progression, growth, metastasis, mammalian target of rapamycin complex 1 (mTORC1)-mediated activation via amino acids. Studies indicated that miR-145 inhibits expression of HBXIP mRNA and HBXIP protein expression correlating with decreased cell proliferation and colony formation in breast tumourigenic cell lines (MCF-7). This negative correlation between HBXIP and miR-145 was confirmed with breast cancer samples. This suggests that miRNA might be a promising target for future breast cancer treatment (Jiang 2019).

It has been reported that glycolytic inhibitors that target hexokinase namely 3-bromopyruvate and 2-deoxyglucose exert anticancer activity in animal tumour models (Pelicano 2006). 3-Bromopyruvate binds to hexokinase II resulting in dissociation of hexokinase from the mitochondrial membrane leading to apoptosis induction via cytochrome *c* release and caspase 3 activation (Wieman 2007). Furthermore, 3-bromopyruvate reportedly demonstrates antitumour activity against several types of cancer including breast, prostate, pancreas, cervix, renal, ovarian, colorectal, hepatic, melanoma and lung (Schmidt 2010).

PI3K/Akt signaling regulates glucose uptake by means of GLUT1 membrane localization. Akt promotes transcription and plasma membrane localisation of GLUT1, thus increasing the rate of glycolysis (Schmidt 2010). Studies show an increase in Akt signaling in nutrient-deprived human breast cancer cells. Inhibition of Akt signaling caused a decrease in cell viability in nutrient-deprived breast cancer cells substantiating the importance of Akt in cell survival (Sudhagar 2016). Akt/mTOR signaling is also implicated in the tumour suppressor activity via miR-150-5p which has been implicated in colorectal cancer, glioma, pancreatic cancer and melanoma where downregulation of miR-150-5p is associated with poor prognosis. Furthermore, where miR-150-5p is overexpressed, phosphorylation and activation of Akt are significantly inhibited accompanied with reduced proliferation- and migration and G1/S arrest in rhabdoid (kidney) tumourigenic cell lines G401 and WT-CLS_1 (Yang 2019). In uterine leiomyoma cells transfection with miRNA-150 mimic resulted in decreased cell numbers, migration and decreased expression of pAkt accompanied with increased expression of an upstream target, cyclin-dependent kinase inhibitor 1B ($p27^{Kip1}$). Akt is capable of reducing $p27^{Kip1}$ expression by means of several modes including loss of

phosphatase and tensin homolog (PTEN) function, impaired nuclear import of $p27^{Kip1}$ and phosphorylation of $p27^{Kip1}$(Lee 2019).

Another miR that has a similar effect on cancer cells is miR-140-5p which shows an inverse correlation with prognosis in nephroblastoma patients where higher tumour grade and least favourable outcome in Wilm's tumour patients correlated with lower miR-140-5p expression (Liu 2019). Tumour expression activity of miR-140-5p has been observed in gastric cancer, hepatocellular cancer, chronic myeloid leukemia and hypopharyngeal cancer. Transfection with miR-140-5p mimic in rhabdoid kidney tumour cell lines G_{401} and WT-CLS_1 resulted in significantly reduced cell growth, inhibition of migration and G_1/S arrest. Further data demonstrated that transfection with miR-140-5p mimic resulted in decreased Akt activation as shown by reduced pAkt and inhibition of transforming growth factor beta receptor 1 (TGFBR1) in G_{401}- and WT-CLS_1 cell lines (Liu 2019). Studies in chronic myeloid leukemia cell lines (K562 and KCL22) transfected with miR-140-5p mimic resulted in significant induction of apoptosis and decreased cell viability and transfection with miR-140-5p mimic led to reduced expression of SIX1. The miR-140-5p/SIX1 axis is responsible for regulation of pyruvate kinase isomer 2 (PKM2) expression whereby transfection with miR-140-5p mimic demonstrated significantly decreased protein PKM2 expression (Nie 2019).

Activity of PKM determines the fate of glucose-derived carbon and is essential in glycolysis (Morita 2018). PKM is an essential enzyme involved in glycolysis that catalyzes the transfer of high energy phosphate of phosphoenolpyruvate to pyruvate while generating ATP (Morita 2018; Hatami 2019). PKM gives rise to two different splice variants encoding PKM1 and PKM2 depending on the alternative mRNA splicing to include exon 9 or exon 10. Both

variants catalyze the same reaction; however, PKM1 possesses constitutively high catalytic activity and PKM2 is allosterically regulated (Hills 2019). Moreover, PKM2 can either exist in a low energy state where it enhances biosynthetic needs or in a high energy state where it promotes oxidative glucose metabolism and thus, PKM2 promotes tumour metabolism, proliferation and survival of tumourigenic cells (Hills 2019; Ye 2019). Conversion of PKM1 to PKM2 shifts glucose metabolism toward aerobic glycolysis, a metabolic phenotype that is amenable for promoting favourable energetics, biosynthetic intermediates and redox power for rapidly dividing tumour cells (Zhao 21019). Overexpression of PKM2 is frequently found in a variety of tumours including colon- and breast cancer and is also associated with poor prognosis in signet ring cell gastric cancer and oesophageal cancer (Desai 2014). Additional studies showed that mRNA levels of PKM2, but not PKM1, were overexpressed on tumourigenic colon tissue and when compared to non-tumourigenic colon tissue (Kim 2019).

PKM2 activity determines breakdown of glucose to lactic acid and silencing of PKM2 resulted insignificantly reduction of glucose consumption, decreased lactic acid production, downregulated GLUT1 expression and inhibition of proliferation in breast tumourigenic cell lines (MCF-7 and MDA-MB-231) (Yao 2019). In addition, inhibition of PKM2 upregulates consumption of oxygen and reduces glucose uptake and lactate production while restoration of PKM2 increases cell proliferation and tumour formation in immunodeficient mice (Yokoyama 2018). Furthermore, overexpression of PKM2 is usually accompanied with the upregulation of the oncoprotein, cellular myelocytomatosis (c-Myc), which activates transcription of heterogeneous nuclear ribonucleoproteins (hnRNPs) I, A1 and A2, which bind and repress exon 9 encoding RNA sequences. Thus, c-Myc promotes preferential splicing of PKM2 mRNA, which allows for the synchronous expression of the PKM2

isoform (Zhao 2019). Studies have indicated that PKM2 regulates cyclin D- and c-Myc expression by means of phosphorylating histone H3 at threonine 11 (H3-T11) resulting in G1-S transition, chromosome segregation, cell cycle progression and thus promotes tumourigenesis (Yokoyama 2018).

Warburg reported that, together with the aerobic glycolysis theory, there is an incident of mitochondrial damage (Jose 2011; Cazzaniga 2015). However, subsequent studies indicated that mitochondrial respiratory function is not inactivated in cancer cells, but rather operates at a low capacity ensuring supply of cellular components (Cazzaniga 2015). Literature has shown that a glycolytic inhibitor, iodoacetate, significantly decreased intracellular ATP production (>90%) in lung epidermoid carcinoma cell lines. However, addition of OXPHOS inhibitors, oligomycin and atractyloside, had a minimal effect (<10%) on ATP reduction suggesting that mitochondrial OXPHOS contributes significantly less in energy production in tumour cells when compared to glycolysis (Bellance 2009).

Research surrounding the role and activity of the mitochondria and OXPHOS is still emerging and presents with a complicated history. There are numerous studies that support the notion that tumourigenic cells prefer glycolysis over OXPHOS. Furthermore, several types of cancer including bladder, breast and kidney are depleted of mitochondrial deoxyribonucleic acid (DNA) and exhibit decreased expression of respiratory genes (Yao 2019). However, an essential regulatory role for wild-type p53 ($p53^{WT}$) has also been identified in cancer metabolism. Recent studies revealed that, in normoxia conditions, $p53^{WT}$ upregulates OXPHOS without affecting glycolysis, resulting in OXPHOS providing more than 70% of ATP. Conversely, under sustained hypoxic conditions, $p53^{WT}$ significantly reduces OXPHOS, whereas glycolysis is not significantly affected, leading glycolysis to

provide more than 60% of the cellular ATP. Thus, additional research is required to provide insight on the molecular determinants regarding the role of the mitochondria in cancer metabolism and this will also be elaborated on in the review as well (Hernández-Reséndiz 2019).

Microenvironment

The tumourigenic microenvironment is acidic due to the high glycolytic rate resulting in accumulation of intracellular lactate and H^+ ions. Lactate and H^+ ions exit the cell into the extracellular fluid resulting in an acidic extracellular environment (Stubbs 2000). The acidic microenvironment is a specialised niche that is highly beneficial to tumourigenic cells. The low pH promotes proliferation, metastasis and glycolysis by several mechanisms including upregulating sodium-hydrogen exchanger-1 and carbonic anhydrase which both play an essential role in regulating pH homeostasis (Chiche 2009; Wojtkowiak 2012; Estrella 2013). In addition, extracellular acidity decreases the intracellular concentration of chemotherapeutic drugs including anthraquinones and vinca alkaloids mostly by ion trapping mechanisms. Ion trapping takes place according to ion protonation of the compounds and results in drug-resistance. Therefore, the acidic microenvironment is a differential target for anticancer compounds that obliterates tumourigenic cells (Pippicelli 2017).

The acidic tumour microenvironment promotes local invasion where the H^+ ions are transported into adjacent non-tumourigenic cells via the concentration gradient. The increase in extrusion of H^+ ions results in an alkaline intracellular pH that drives oxidative phosphorylation. However, increased uptake of H^+ ions promotes a more acidic intracellular

environment that upregulates glycolysis (Amith 2017). Unlike tumourigenic cells, non-tumourigenic cells cannot tolerate the acidic microenvironment (Estrella 2013). The unfavourable acidic environment in non-tumourigenic cells results in the degradation of the extracellular matrix due to activity of proteinases, increase in VEGF and inhibition of immune response to tumour antigens resulting in migration of cells and subsequent metastasis (Fukumura 2001; Estrella 2013).

The acidic microenvironment has been associated with promoting tumourigenesis and tumour cell dormancy since the acidic pH increases resistance to induction of apoptosis and autophagy, increases angiogenesis, promotes tumour cell invasion and obscures immune surveillance (Halcrow 2019). The high level of acidity also inhibits T cell-mediated immune surveillance whereby the lactic acid secretion by T lymphocytes is inhibited of both innate- and adaptive immunity (El-Kenawi 2018, Wong 2019). Subsequently, the acidified T lymphocytes experience reduced metabolism and acidosis, while the low-pH environment indirectly impairs cytokine production and secretion; both effects result in local immunosuppression (Wong 2019).

Decreased pH also promotes the activity of tumour-associated macrophages (TAMS) (El-Kenawi 2018). TAMs promote growth, invasion and metastasis of several types of cancer including lung cancer, breast cancer, and the hepatocellular carcinoma. Recent studies have indicated that TAMs accomplishes this in salivary adenoid cystic carcinoma by means of the monocyte chemotactic protein-1 (MCP-1 or CCL2)/C-C chemokine receptor type 2 (CCR2) axes with further suggestion that the CCL2/CCR2 axis is also responsible for the M2

polarization which is associated with aggressive behaviour of malignant tumours (Yang 2019).

Hypoxia is a recognised characteristic of the specialised niche environment that is beneficial for tumourigenesis and responsible for failed responses to anticancer treatment (Colegio 2014). Hypoxia-regulated pathways including HIF expression regulate the acidic microenvironment. Hypoxia induces the activation and stabilization of HIF's that subsequently results in the expression of membrane-bound enzymes such as carbonic anhydrase IX and XII (Böhme 2016). In addition, hypoxic conditions also present with induction of miR-210 expression in several types of tumours including ovarian serous cystadenocarcinoma, colon adenocarcinoma and rectum adenocarcinoma. Expression of miR-210 also positively correlates with lactate dehydrogenase A (LDHA) upregulation in breast- and ovarian cancer which is indicative of hypoxia-associated metabolic changes (Bhandari 2019). Studies have also shown that miR-210 is an essential factor involved under hypoxia and targets HIF-1α. HIF-1 consists of HIF-1α- and HIF-1β constitutes. Under normoxic conditions HIF-1α associates with the von Hippel-Lindau E3 ligase complex and then subsequently undergoes ubiquitination and proteasomal degradation. However, hypoxic conditions inhibit HIF-1α degradation and results in HIF-1α stabilization which upregulates miR-210 expression and enhances invasion and metastasis of tumourigenic cells (Li 2019).

Multiple studies have shown that the hypoxia microenvironment promotes tumourigenesis, metastasis and resistance to anticancer therapy including chemotherapy, radiotherapy and photodynamic therapy (PDT). Thus, research has also focused on processes and anticancer compounds that reduce hypoxia including hyperbaric oxygen therapy and oxygen-generating

compounds (MnO_2, catalase and perfluorohexane) (Xu 2018). MnO_2-based nanoparticle therapy, poly(lactic-co-glycolic acid) (PLGA)-catalase-based nanoparticle and $MnFe_2O_4$ nanoparticle are dependent on catalysing endogenous hydrogen peroxide in order to generate oxygen while perfluorohexane-based oxygen carriers transport oxygen to the tumourigenic tissue (Xu 2018). Novel compounds based on the same principle include cerium oxide nanoparticles due to owning enzyme-like activities similar to catalase with the ability to reversibly switching from Ce^{4+} to Ce^{3+} while converting hydrogen peroxide to water and oxygen. Studies have also shown significant benefit when used in conjunction with PDT and chemotherapy. However, concerns with cerium oxide and PDT include the optical damage and limited penetration distance in the tissue when directly illuminated by ultraviolet light (Yao 2018).

Carbonic anhydrase IX is responsible for catalysing the reversible hydration of carbon dioxide to carbonic acid. The latter promotes acidification of the extracellular environment and contributes to maintaining an alkaline intracellular pH. This was confirmed when Madin-Darby Canine kidney (MDCK) cells, that do not express endogenous carbonic anhydrase IX, were transfected to constitutively express human carbonic anhydrase IX protein. These MDCK cells were then cultured in hypoxic conditions resulting in a significantly more acidic extracellular environment and an alkaline intracellular environment when compared to MDCK cells that weren't transfected and didn't express carbonic anhydrase IX (Chaabane 2013). Carbonic anhydrase thus adjusts the cells to hypoxia and increases extracellular acidity, proliferation and metabolism (Gupta 2012). Inhibition of carbonic anhydrase IX results in cell death induction, decreased cell viability, compromised cell migration *in vitro.* *In vivo* findings of carbonic anhydrase IX inhibition in murine models indicate abrogated tumour formation and decreased metastasis (Glasauer 2014).

Hypoxic conditions and the low extracellular pH present in tumours induce VEGF messenger ribonucleic acid (mRNA) expression and protein synthesis in human glioblastoma cells (Xu 2002). VEGF is associated with the promotion of angiogenesis, tumour growth, invasion and metastasis (Tang 2016). Colon adenocarcinoma (LS174), melanoma (Mu89) and glioma (U87 MG) cells all exhibited an increase in VEGF expression when cultured in acidic conditions (Fukumura 2001). This is due to a proton-coupled symporter, monocarboxylate transporter (MCT), which regulates lactate production and excretion (Colegio 2014). Fourteen families of MCTs have been identified thus far. MCT1 and MCT2 are responsible for lactate uptake and oxidation. MCT4 regulates lactate production resulting from glycolysis and promotes proliferation and cell migration (Whitworth 2017). Furthermore, MCT4 itself is upregulated in hypoxic acidic conditions via a HIF-dependent mechanism. Thus, MCT-4 protects the cell against lethal lactate quantities that gets produced in tumourigenic upregulated glycolysis (Lu 2013).

Mitochondrial function

Mitochondria are essential bioenergetics- and signaling organelles that are crucial in reacting to cellular stress, hypoxia and acidity in the microenvironment. Furthermore, mitochondria play a key role in tumourigenesis since it is an indispensible organelle for ATP production and adaptions to cellular- and environmental changes due to cancer treatments. Mitochondrial biogenesis, turnover, fission, apoptosis induction, oxidative stress and cell signaling are integral to how tumourigenesis and drug resistance is promoted (Vyas 2016).

In addition to increased metabolism and glucose fermentation in the presence of oxygen, Warburg also suggested that tumourigenic cells possess defective mitochondrial respiration (Boland 2013). Decades later we now know that mutation genes encoding for p53, the MYC oncogene and PI3K are responsible for the upregulation of glycolysis in tumourigenic cells; however, the mitochondrial functioning is not defective (Zong 2016). Despite increased glycolysis, tumourigenic cells still produce a significant amount of energy via mitochondrial respiration (LeBleu 2014). Furthermore, recent studies also indicate that mitochondrial metabolism is not completely diminished in cancer cells, but might exhibit significantly low to even high activity in cancer cells. This data suggests the possibility that different cell states during the process of carcinogenesis utilize disparate fuel sources to keep up with increased metabolic demands (Hirpara 2018).

Studies have demonstrated OXPHOS inhibition in tumourigenic cells resulted in impaired ability for anchorage-independent proliferation and increased sensitivity to cytotoxic drugs. Impairment of mitochondrial deoxyribonucleic acid (mtDNA) results in diminished OXPHOS function and increased ROS levels leading to DNA mutations that promote tumour growth by activation of Akt and PI3K cell survival pathways (Hsu 2013; Sullivan 2014). This indicates that, although tumourigenic cells largely depend on glycolysis for ATP production, mitochondrial respiration is still essential for the fully tumourigenic phenotype exhibited by tumourigenic cells (Viale 2015).

Although cancer cells are reliant on glycolysis for energy production, several cancer cell lines including pancreatic ductal adenocarcinoma have been identified to be dependent on OXPHOS for energy production, proliferation and metastasis (Rademaker 2019). Tumour

subtypes that prefer OXPHOS are characterised by upregulation of genes encoding the respiratory chain components accompanied with increased mitochondrial respiration and enhanced antioxidant defences (Gentric 2019). Furthermore, tumours presenting with metabolic plasticity can switch between oxidative metabolism to glycolysis and *vice versa*. However, this is dependent on genetic- and epigenetic regulation and is contingent on several factors including time- and dose of exposure to biological- and chemical agents, environmental change and magnitude of the mitochondrial- or glycolytic inhibition (Christie 2019).

Conflicting data exists pertaining to increased mitochondrial activity that has been associated with poor clinical outcome in several types of tumours where metastasis to distant sites are often observed and correlates with epithelial-to-mesenchymal transition (EMT) gene patterns (Lunetti 2019). Furthermore, cancer cells in circulation have increased oxidative phosphorylation and invasive breast cancer cells demonstrate a shift toward OXPHOS metabolism via peroxisome proliferator-activated receptor gamma coactivator 1-alpha **(**PGC-1α**)** which is an essential regulator of mitochondrial biogenesis and metabolism (Liu 2019). Literature has shown that downregulation of OXPHOS-related genes correlate with metastasis and poor prognosis. A basal breast tumourigenic cell line (MDA-MB-231 cell line) presents with decreased mitochondrial respiration and reduced expression of succinate dehydrogenase B, which is the core catalytic subunit of the mitochondrial heterotetrameric complex succinate dehydrogenase, which is essential for OXPHOS. Decreased expression of succinate dehydrogenase B has been associated with reprogramming carbon source utilization and aberrant mitochondrial activity which results in cell migration and EMT (Lunetti 2019).

Thus, the mitochondrial metabolism and OXPHOS represent a potential target for future anticancer treatments for several types of cancer including pancreatic ductal adenocarcinoma, lung cancer and colon cancer (Liu 2019). Dicholoroacetate, a compound that shifts metabolism from anaerobic glycolysis to OXPHOS by means of stimulating pyruvate dehydrogenase kinase (PDH), exerted antiproliferative activity, decreased viability in pancreatic PANC-1 pancreatic carcinoma-derived cell line and pancreatic adenocarcinoma-derived cell line BxPC-3. In addition, dicholoroacetate reduced the growth of PANC-1- and BxPC-3 spheroids models and reduced the tumor sizes by 25-30% in pancreatic cancer xenografts mouse models (Tataranni 2019).

Due to increased tumourigenic metabolic activity in order to support hyperproliferation, tumourigenic cells produce increased quantities of ROS via the mitochondria and endoplasmic reticulum when compared to non-tumourigenic cells (Fulda 2010). ROS has multiple signaling responsibilities in cell activities including proliferation and apoptosis induction. The effects induced by ROS are dependent on the type of ROS in question, amount of ROS and the location thereof. Furthermore, these regulated ROS quantities depend on the type of ROS, the cell line, the inducer and the antioxidant defence system used for that specific ROS type ROS acts in a biphasic manner and at regular cell redox quantities exerts pro-survival signaling. This is confirmed by research where ROS scavengers in high doses abrogate proliferation in mammalian cell cultures (Chaabane 2013; Scott 2014; Garrido-Maraver 2015). However, damage to the endogenous antioxidant systems of the cells or other cell stress including radiation leads to oxidative DNA damage and nucleic mutations in the mitochondria ultimately resulting in apoptosis induction (Scott 2014). Thus, ROS regulate cell number by either activating pro-survival signaling or induces apoptosis (Chaabane 2013).

In tumourigenic cells, ROS activate transcription factors including nuclear factor kappa light-chain enhancer of activated B cells (NF-κB), HIF-1α and signal transducer and activator of transcription 3 (STAT3) which regulate cell transformation, survival, proliferation, invasion, angiogenesis and metastasis (Gupta 2012; Glasauer 2014). Moderate levels of ROS increase proliferation of breast cancer cells by recruiting cells in the S phase of the cell cycle, thus enhancing the expression of MYC and increasing NF-κB activity (Gupta 2012).

Electrons from the electron transport chain in the mitochondria leak out to molecular oxygen to subsequently produce superoxide. Superoxide enters the cytoplasm via the pores in the mitochondrial outer membrane resulting in cytochrome *c* release, apoptosome formation and activation of caspases culminating in apoptosis (Gupta 2012). Mitochondrial damage also results in cytochrome *c* release into the cytosol which signals for apoptosis (Gupta 2012; Glasauer 2014). Excessive ROS results in increased vulnerability of mitochondrial membrane permeability transition which can potentially lead to cell death unless mitophagy takes place. Mitophagy is the selective degradation of dysfunctional or impaired mitochondria, which along with mitochondrial biogenesis, regulate mitochondrial mass degradation and formation (Garrido-Maraver 2015). Mitophagy thus promotes turnover of damaged or defective mitochondria that would otherwise be detrimental to the cell. However, how the cell distinguishes between healthy and damaged mitochondria remains unknown. Mitochondrial fragmentation and loss of mitochondrial membrane potential precede mitophagy indicating that these factors might be integral to how mitochondria are selected for mitophagy (Garrido-Maraver 2015; Chourasia 2015).

Mitophagy stimuli include hypoxia, nutrient deprivation, oxidative stress, DNA damage, inflammation and mitochondrial membrane depolarization (Drake 2017). The mechanism of action utilized by mitophagy involves interactions with several key adaptor molecules BCL-2/adenovirus E1B 19kDa protein-interacting protein 3 (BNIP3) and FUN14 domain containing 1 (FUNDC1) at the outer mitochondrial membrane with microtubule-associated protein 1A/1B-light chain 3 (LC3) at the phagophore membrane. Mitochondrial targets of E3 ubiquitin ligase including Parkin and Mull are also involved (Chourasia 2015). Parkin regulates mitochondrial morphology and is widely expressed in brain-, skeletal muscle-, heart- and liver tissue. Damaged mitochondria stimulate accumulation of Parkin to the mitochondrial surface which activates a process that results in autophagy of damaged mitochondria (Whitworth 2017). Parkin translocates to the depolarized mitochondria in cells exposed to a mitochondrial uncoupler, carbonyl cyanide m-chorophenyl hydrazine (CCCP), abolishes the mitochondrial membrane potential and induces mitochondrial damage (Lu 2013). Novel findings also report that Parkin is an essential tumour suppressor in breast-, liver-, bladder-, lung- and ovarian cancer (Lu 2013; Boland 2013; Palikaras 2016). Mice lacking Parkin develop liver tumours and are vulnerable to irradiation-induced lymphomagenesis (Palikaras 2016).

Damaged mitochondria are duplicated in tumours which may be beneficial for tumour progression (Boland 2013). Studies have shown that mutations in the mitochondrial genome can add to the development of chemoresistance in malignant tumours (Guaragnella 2014). Several anticancer agents namely arsenic trioxide decrease glutathione and increase the production of hydrogen peroxide and superoxide which result in the induction of ROS-dependent cell death (Kang 2004). Literature has shown that anticancer drugs namely betulinic acid and celastrol, stimulate permeabilization of mitochondrial outer membrane and

inhibited mitochondrial respiratory chain complex I in lung cancer cells respectively (Kasiappan 2016). Prasad et al (2016) demonstrated that regosertib, a stryryl benzyl sulfonate, inhibited Akt/PI3K/mTOR pathway, induced increased ROS production and activated of c-Jun N-terminal kinase or stress-activated protein kinase (JNK)-dependent cell death induction in head and neck squamous cell carcinoma cells, thus resulting in apoptosis and attenuation of cell cycle progression (Prasad 2016).

Mitochondrial biogenesis, a process whereby new mitochondria are produced is usually coupled with mitophagy. When defective mitochondria are destroyed, new ones are produced (Zhu 2013). Several signaling pathways regulate mitochondrial biogenesis including estrogen-related receptor α (ERRα)/peroxisome proliferator-activated receptor gamma coactivator-1 (PGC1) pathway (mitochondrial biogenesis regulating pathway), protein kinase A (PKA), peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PPARGC1A), mitochondrial transcription factor A (TFAM), mitogen-activated protein kinase (MAPK) 1 and MAPK3. Markers of mitochondrial biogenesis are decreased in cancer. Decreased expression of PPARGC1A and TFAM is observed in lung cancer (Zhu 2013). TFAM, oxidative phosphorylation components and mtDNA present in cervical carcinoma, osteosarcoma and breast cancer cells resulted in decreased proliferation and inhibited migration (Wang 2011). Exposure to an inhibitor of ERRα-PGC1 pathway, XCT790, in MCF-7 breast cancer cell lines demonstrated that XCT790 blocks the survival and progression of tumour initiating stem-like cells (De Luca 2015). Recent studies are investigating mitochondrial repair mechanisms including mitophagy and mitochondrial biogenesis to overcome mitochondrial related diseases including cancer (Whitaker 2013; Garret 2014). Stressors such as nutrient deprivation results in mitophagy (activated by AMPK) and mitochondrial biogenesis through the activation of PGC-1 induced by AMPK

(Palikaras 2014). It is crucial to have a balance between mitochondrial biogenesis and mitophagy since an imbalance could result in various human pathologies (Xiong 2019). In the light of the above-mentioned research has presented a promising future in ROS-inducing chemotherapeutic agents in combination with anticancer drugs (Kryeziu 2016).

Conclusion

Since the Warburg effect was first described, it has been a major focus in cancer research and, although new information regarding metabolism in tumourigenic cells is emerging, Warburg's theory still applies where tumourigenic cells prefer glycolysis rather than mitochondrial OXPHOS (Lu 2015). Glycolytic tumours increase their glucose uptake by upregulation of GLUTs and release H^+ ions into the extracellular environment making it a favourable condition to proliferate and invade other cells (Stubbs 2000).

Tumourigenic cells have a neutral intracellular pH and an acidic extracellular environment which they have adapted to. The acidic microenvironment promotes invasion into adjacent resulting in malignant transformation and distal metastasis (Huang 2016). Acidic extracellular environment in tumours decreases the efficacy of chemotherapeutic drugs, thus research investigating drugs that target and alter the microenvironment is of importance (Stubbs 2000). With a full understanding of the mechanisms of tumour cell metabolism, research can be conducted to target those mechanisms in order to antagonise tumour cell pathways and improve chemotherapeutic differential targeting mechanisms.

Mitochondria play a vital role in energy production and the production of cellular biomass, therefore, damaged mitochondria may result in low energy production and the inability to respond to the cells' demand for energy ultimately leading to damaged cellular structures and increased pathologies linked to mitochondrial dysfunction (Thiessen 2015). Mitochondrial repair by mitochondrial biogenesis and mitophagy can be used for the recovery of damaged mitochondria and the recovery of cellular functioning (Zhu 2013). Upregulation and recruitment of mitochondrial biogenesis regulators has shown to restore the mitochondria, this could also be incorporated in combating mitochondrial dysfunction (Sheng 2012). Research has shown that inhibiting the mitochondrial biogenesis pathway decreases survival and attenuates progression of tumours (De Luca 2015). Unravelling the complex mitochondrial dynamics in cancer will provide scientists and clinicians with improved understanding of how cancer proliferates, migrates and is resistant to chemotherapy and radiation as well as their potential in identification of novel biomarkers for damaged or manipulated mitochondria in tumourigenesis.

Conflict of interest

The authors declare no conflict of interest.

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