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The key role of Warburg effect in SARS-CoV-2 replication and associated inflammatory response

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Current mortality due to the Covid-19 pandemic (approximately 1.2 million by November 2020) demonstrates the lack of an effective treatment. As replication of many viruses - including MERS-CoV - is supported by enhanced aerobic glycolysis, we hypothesized that SARS-CoV-2 replication in host cells (especially airway cells) is reliant upon altered glucose metabolism. This metabolism is similar to the Warburg effect well studied in cancer. Counteracting two main pathways (PI3K/AKT and MAPK/ERK signaling) sustaining aerobic glycolysis inhibits MERS-CoV replication and thus, very likely that of SARS-CoV-2, which shares many similarities with MERS-CoV. The Warburg effect appears to be involved in several steps of COVID-19 infection. Once induced by hypoxia, the Warburg effect becomes active in lung endothelial cells, particularly in the presence of atherosclerosis, thereby promoting vasoconstriction and micro thrombosis. Aerobic glycolysis also supports activation of pro-inflammatory cells such as neutrophils and M1 macrophages. As the anti-inflammatory response and reparative process is performed by M2 macrophages reliant on oxidative metabolism, we speculated that the switch to oxidative metabolism in M2 macrophages would not occur at the appropriate time due to an uncontrolled pro-inflammatory cascade. Aging, mitochondrial senescence and enzyme dysfunction, AMPK down-regulation and p53 inactivation could all play a role in this key biochemical event. Understanding the role of the Warburg effect in COVID-19 can be essential to developing molecules reducing infectivity, arresting endothelial cells activation and the pro-inflammatory cascade.

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1. Introduction

Coronavirus SARS-CoV-2 is responsible for the Coronavirus Disease 2019 (COVID-19), a viral pandemic which has resulted to approximately 1.2 million deaths worldwide as of early November 2020. Mortality rates are higher among elderly patients, especially those suffering from hypertension, obesity, diabetes, metabolic syndrome, cardiac or renal failure [1]. Available anti-viral drugs may target all stages of virus replication, from viral cell entry to the release of new viruses, but none of them have appeared to be effective. Therefore, finding more efficient strategies is urgently needed.

Considering that many viruses induce metabolic reprogramming in host cells in a similar way to the Warburg effect in cancer cells (i.e. enhancement of glycolysis with lactate production) [2–6],
List of abbreviations

2-DG 2-deoxy-D-glucose  
ACC acetyl-CoA carboxylase  
ACE2 angiotensin-converting enzyme 2  
Acetyl-CoA acetyl coenzyme A  
ACLY ATP citrate lyase  
ACO2 mitochondrial aconitase  
Acyl-CoA acyl coenzyme A  
AKG a-ketoglutarate  
AKT Protein kinase B  
AMPK AMP-activated protein kinase  
Ang angiotensin  
ARDS acute respiratory distress syndrome  
ARG1 arginase 1  
ATF3 activating transcription factor  
CAD cis-Aconitate decarboxylase  
CD36 cluster of differentiation 36  
COVID-19 Coronavirus disease 2019  
DCs dendritic cells  
EGF epidermal growth factor receptor  
ERK extracellular signal-regulated kinase  
F1,6BP fructose 1,6-bisphosphate  
F6P fructose 6-phosphate  
FAO fatty acid oxidation  
FAS fatty acid synthesis  
GLS1 Glutaminase 1  
Glut glutamate  
GLUT glucose transporter  
HCMV human cytomegalovirus  
HIF-1a hypoxia-inducible factor-1 alpha  
IDH isocitrate dehydrogenase  
Il1 interleukins  
INOS inducible NO synthase  
LDHA lactate dehydrogenase A  
MALA malonyl-coenzyme A  
MAPK mitogen-activated protein kinase  
MCT monocarboxylate transporter  
MERS-CoV Middle East Respiratory Syndrome coronavirus  
MOF multi-organ failure  
mTOR mammalian target of rapamycin  
MYC Myelocytomatosis Viral oncogene  
NADPH,H+ nicotinamide adenine dinucleotide phosphate  
NK natural killer  
NO nitric oxide  
NRF2 nuclear factor erythroid 2 p45-related factor 2  
OAA oxaloacetate  
OXPHOS oxidative phosphorylation  
PAH pulmonary arterial hypertension  
PC pyruvate carboxylase  
PDGF platelet-derived growth factor  
PDH pyruvate dehydrogenase  
PDK1 pyruvate dehydrogenase Kinase 1  
PK1 phosphofructokinase1  
PK3 Phosphoinositide 3-kinase  
PK pyruvate kinase  
PKM2 pyruvate kinase muscle isozyme 2  
PPP pentose phosphate pathway  
R5P ribose 5-phosphate  
RAS rat sarcoma viral oncogene homolog  
ROS reactive oxygen species  
SARS-CoV-2 severe acute respiratory syndrome coronavirus 2  
SDH succinate dehydrogenase  
SGLT1 sodium glucose cotransporter 1  
SOD2 superoxide dismutase 2  
TCA tricarboxylic acid cycle  
TGEV transmissible gastroenteritis virus  
TNF-α tumor necrosis factor-α  
VSMC vascular smooth muscle cells  
ZIKV Zika virus

we examined the hypothesis that glycolytic metabolism also supports SARS-CoV-2 replication in airway cells, namely type I and type II pneumocytes which represent more than 95% of the alveolar surface [7].

Furthermore, we provide arguments supporting a role for the Warburg effect’s involvement in several steps of COVID-19 infection, as hypoxia promotes aerobic glycolysis, in particular in the endothelial cells, especially in the presence of atherosclerosis. Moreover, we will discuss how the Warburg effect supports activation of pro-inflammatory macrophages and cytotoxic immune cells against pathogens [8]. Finally, we will speculate that the switch to oxidative metabolism in macrophages implied in the anti-inflammatory response is altered and/or does not occur at the appropriate time in severe COVID-19 disease. As we will see, this switch can be altered by many factors such as chronic hypoxia, mitochondrial senescence, enzymatic dysfunctions or deregulations, AMPK and p53 inactivation. Understanding the Warburg effect in this broader perspective can be essential in developing new therapeutics for reducing infectivity and mortality.

2. The Warburg effect sustains the metabolism and replication of numerous cell types

In order to replicate, numerous cell types (cancerous or not) increase their nutrient consumption (in particular glucose and glutamine). At the beginning of the 20th century, Otto Warburg first observed that cultured cancer cells have a high rate of glycolysis and secrete lactate, even in the presence of oxygen (O2) [9]. This so-called aerobic glycolysis or “Warburg effect” has been extensively studied in cancer cells over the past years, and thus a brief presentation will facilitate further comprehension of its possible role in COVID-19.

2.1. The Warburg effect in cancer cells

Aerobic glycolysis promotes cancer cells invasiveness, aggressiveness, and drug resistance [10,11]. The shift from oxidative metabolism to increased glycolysis with lactate production is promoted by hypoxia and the hypoxia-inducible factor-1 alpha (HIF-1α) [12]. This switch is related to pyruvate dehydrogenase (PDH) inhibition by pyruvate kinase dehydrogenase 1 (PDK1), a process stimulated by HIF-1α, phosphatidylinositol 3-kinase (PI3K)/protein kinase B (PI3K/AKT) and the mitogen-activated protein kinases/extracellular signal-regulated kinase (MAPK/ERK) signaling pathways [13–15]. These key signaling pathways are activated by loss of suppressors such as P53, and activation of oncogenes such as Rat Sarcoma Viral oncogene homolog (RAS) and Myelocytomatosis Viral oncogene (MYC) [16]. Importantly, it is noteworthy that together with promotion of the Warburg effect [17], PI3K/AKT pathway and phosphorylated sugars activate ATP citrate lyase (ACLY), thus linking enhancement of aerobic glycolysis and acetyl-CoA production [for references, see Ref. [18]. Acetyl-CoA molecules sustain de novo
fatty acid synthesis (FAS) required for membrane formation. Lactate derived from pyruvate is expelled in the microenvironment (ME) promoting tissue acidity and immune cells exhaustion [19].

Glycolysis and its branched metabolic pathways (such as the pentose phosphate pathway (PPP) and the serine pathway) sustain the production of molecules composing nucleotides (such as ribose 5-phosphate (RSP), glycine and methyl groups). PPP furnishes also nicotinamide adenine dinucleotide phosphate (NADPH), a reduced cofactor required for FAS and reduct system. As mitochondrial tricarboxylic acid (TCA) cycle functioning is down-regulated by the Warburg effect, cancer cells often increase glutamine metabolism to reload the TCA cycle into α-ketoglutarate (AKG), thus furnishing molecules for biosynthesis. Glutamine metabolism also provides nitrogen groups for nucleotide synthesis and can sustain the ACLY reaction, in particular via a reversed isocitrate dehydrogenase (IDH) route [10,20]. As the Warburg effect and glutamine metabolism concomitantly sustain FAS and nucleotides synthesis, they appear as key targets for cancer cells inhibition [21].

2.2. The Warburg effect likely sustains the replication of SARS-CoV-2 in airway cells

Many viruses alter the host cell metabolism in a similar way to the Warburg effect, enhancing glycolysis and therefore producing rapid energy and “bricks” for nucleotide replication and specific protein synthesis [2,6]. Enhanced aerobic glycolysis has been shown in Zika virus (ZIKV) with up-regulation of glycolytic genes, including membrane glucose transporter 1 (GLUT1), several enzymes of glycolysis, and MCT4, the transporter expulsing lactate outside cells [6]. During infection, human cytomegalovirus (HCMV) increases the expression of glucose transporter 4 (GLUT4) which has a higher glucose transport capacity than GLUT1 [22]. In intestinal cells, the coronavirus transmissible gastroenteritis virus (TGEV) increases glucose absorption via the apical transporters Na+-dependent glucose transporter 1 (SGLT1), and the basal glucose transporter 2 (GLUT2), an uptake stimulated by the epidermal growth factor receptor (EGFR) [23].

Host cells metabolism and activation of signaling pathways is reprogrammed by viral proteins as showed in adenovirus-E4-ORF1 [24]. The two key signaling pathways - PI3/AKT/mTOR and MAPK/ERK - promote the replication of Middle East Respiratory Syndrome coronavirus (MERS-CoV) [5], enterovirus 71 (EV71) [25] and ZIKV [26]. In MERS-CoV, PI3/AKT/mTOR and MAPK/ERK inhibitors (including rapamycin) inhibit the virus replication in vitro, regardless if the inhibitors are introduced prior or after viral infection [5].

Thus, considering that SARS-CoV-2 and MERS-CoV are betacoronavirus which share numerous similarities [27], we hypothesize that SARS-CoV-2 replication is supported in airway cells by the Warburg effect promoted by PI3/AKT/mTOR and MAPK/ERK pathways (Fig. 1). Interestingly, during the reviewing process of this manuscript, a study reported that SARS-CoV-2 replication is supported in colon cancer cells by increasing carbon metabolism, and this replication is inhibited by 2-deoxy-D-glucose (2-DG), a glycolysis inhibitor [28]. However, the scientific demonstration of our hypothesis remains to be tested in airway cells, in which the SARS-CoV-2 virus develops. Such studies need to give priority to the following considerations: 1) that the metabolism may be cell type-dependent; 2) the Warburg effect may be activated independent of the infection itself (as an example in response to hypoxia); 3) and the virus could promote alternative pathways (in particular glutaminolysis) to adapt nutrient conditions to biosynthetic or bienergetic demands. Thus, inhibition solely of glycolysis would be inefficient as a means to inactivate the viral replication.

Importantly, AMP-activated protein kinase (AMPK) is the key sensor of energy in eukaryotic cells which switches off ATP-consuming processes, and promotes oxidative functioning, in particular fatty acid oxidation (FAO) [30]. AMPK is a well-known inhibitor of the Warburg effect and of PI3K/AKT/mTOR [31]. In ZIKV, AMPK attenuates virus replication, inhibits inflammatory mediators such as tumor necrosis factor-α (TNF-α) and up-regulates genes demonstrating antiviral properties [6]. Thus, it is likely that in MERS-CoV, PI3K/AKT/mTOR and MAPK/ERK supporting viral replication, are not efficiently counteracted by AMPK. Thus, AMPK activators could be tested in laboratory studies to prevent MERS-CoV and in the current context, SARS-CoV-2 replication.

3. The Warburg effect may sustain many other aspects of COVID-19 disease

3.1. The Warburg effect is promoted in endothelial cells by hypoxia

Hypoventilation induces local pulmonary arterial vasoconstriction, redirecting the blood flow to better ventilated areas, thus maintaining oxygenation [32]. Pneumonia induces local hypoxia in pulmonary alveoli, a condition which induces the Warburg effect in endothelial cells in close contact with pneumocytes, while local acidity favors interstitial edema and stress of these cells [32]. Furthermore, hypoxia promotes von Willebrand factor and thrombosis in micro-vessels [33]. Importantly, aerobic glycolysis is stimulated by PDK1 (the PDH inhibitor) and ERK in platelets, resulting in thromboxane activation and micro thrombosis [34]. In turn, platelet-derived growth factor (PDGF) activates the Warburg effect via PI3K signaling pathway and HIF-1α [34]. Extensive thrombosis can lead to pulmonary arterial hypertension (PAH) with HIF-1α activation in a feedback loop [35]. Finally, extension of infected lung areas relying on the Warburg effect can lead to severe hypoxia, PAH and acute respiratory distress syndrome (ARDS) [36,37]. It is noteworthy that AMPK likely attenuates von Willebrand factor by counteracting PI3K pathway activated by PDGF, while it may counteract platelet activation by inhibiting acetyl-CoA carboxylase (ACC), the first enzyme of FAS sustaining arachidonic acid synthesis, a pathway crucial for thromboxane generation [38]. Furthermore, AMPK possibly increases the production in endothelium of angiotensin 1–7 (Ang1-7) [39], a vasodilator derived from Ang1-9 secreted by angiotensin-converting enzyme 2 (ACE2) [40]. ACE2 is the membrane receptor of SARS-CoV-2 [41], and cleavage of this receptor by viral proteins increases infectivity but functionally alters ACE2 [42]. Thus, Ang1-9 and Ang1–7 production are impaired, and Ang1–7 does not counteract the vasoconstrictor effect of angiotensin II (Ang II) on lung vessels [43,44]. As AMPK phosphorylates and stabilizes ACE2 [39], it may promote the production of Ang-1–7, thus attenuating the vasoconstrictive effect of Ang II.

In summary, the Warburg effect is promoted in endothelial cells of lung vessels by hypoxia and this activation sustains vasoconstriction and platelet micro thrombosis. PI3K signaling and HIF-1α are activated in a feedback loop. If this cascade is not arrested, in particular by AMPK activation, it can result in extensive lung damage.

3.2. The Warburg effect is promoted by atherosclerosis

Atherosclerosis is a vascular pathology caused by atheroma deposits in the wall of the vessels, narrowing the lumen and restricting the blood flow. This pathological injury, which increases with aging, sustains a chronic inflammatory state of the cardiovascular system [45]. It favors the Warburg effect which sustains proliferation of vascular smooth muscle cells (VSMC) [46]. Atherosclerosis appears as a common comorbidity factor in severe
COVID-19 disease because it is frequently observed in patients with metabolic syndrome, diabetes, obesity, arterial hypertension, cardiovascular disorders and chronic nephropathy [1,47]. All these pathologies promote the occurrence of a low-grade inflammation state, a condition increased with aging ("inflammaging"). This chronic state of inflammation impairs the endothelial function, immune response and antioxidant defense, while it increases development of cancers [48,49].

Aging increases the Warburg effect in cells, in particular via oxidative stress and production of reactive oxygen species (ROS) promoting HIF-1α activation; mitochondrial senescence, decreased oxidative phosphorylation (OXPHOS), dysfunction of AMPK and p53 pathways conspire to promote aerobic glycolysis in elderly patients [47,50].

### 3.3. The Warburg effect supports the pro-inflammatory innate and adaptive immune response

The Warburg effect also sustains defense against bacterial and virus infection. Indeed, aerobic glycolysis supports activation and proliferation of neutrophils and activation of M1 macrophages, all cells involved in the innate defense [8,51]. These cells rely on high consumption of glucose to sustain a rapid and efficient response against microbes with secretion of high amounts of nitric oxide (NO), pro-inflammatory lipid molecules (as eicosanoids), and cytokines, in particular of interleukins (ILs) (as IL-1 and IL-6) and TNF-α [52,53]. Furthermore, aerobic glycolysis sustains the secondary proliferation of cytotoxic and B lymphocytes which have been activated by antigens presented by macrophages and dendritic cells [54]. On the contrary, the activation of the anti-inflammatory response with secretion of IL-10 is mainly supported by M2 macrophages (M2) whose metabolism principally relies on FAO activation [52,53,55]. This efficient way of ATP production sustains the cleaning and recycling processes ensured by M2, in particular autophagy [53] (Fig. 2). Importantly, M1 versus M2 activation is regulated by two enzymatic pathways competing for arginine, namely the inducible NO synthase (iNOS) and the arginase pathways. These pathways inhibit each other, since they are regulated by two opposite signaling: i) the PI3K/AKT/mTOR sustains the iNOS axis in M1 which produces high levels of NO and pro-inflammatory molecules [52,53,56]; ii) the AMPK signaling activates in M2 the Arginase 1 (ARG1) reaction which hydrolyzes L-arginine into urea and ornithine; this latter molecule is a precursor of proline and polyamines, which are involved in tissue repair and wound healing [52,53]. In addition to these opposing functions, there would also be regulation of the TCA cycle exercised at the level of the mitochondrial aconitase (ACO2) [57]: NO inhibits this enzyme, which results in a “truncated” TCA cycle [58], a process reinforced by isocitrate dehydrogenase 2 (IDH2) inactivation [59]. Decarboxylation of aconitate by CAD (cis-Aconitate decarboxylase) (also name as immunoresponsive gene 1 (IRG1), or ACOD1) increases itaconate concentration in mitochondria, resulting in succinate.
dehydrogenase (SDH) inhibition and HIF-1α activation [60]. AC2 and/or IDH2 inhibition promotes citrate mitochondrial export in M1. Acetyl-CoA sustains histone acetylation (promoting transcription of genes sustaining inflammatory response) and also the production of pro-inflammatory lipids.

In contrast, in M2 macrophages, AMPK inhibits FAS (in particular by inactivation of ACC), and citrate feeds the cytosolic production of itaconate. This molecule promotes activation of ATF3 (a negative by inactivation of ACC), and citrate feeds the cytosolic production of arginine, transformed by ARG1 into ornithine required for repairing (proline and polyamine synthesis)

As we have seen, SARS-CoV2 replication is likely supported by the Warburg effect in cells expressing ACE2, particularly in nasal epithelial and pneumocytic cells which represent the body’s airway entry site for the virus. This hypothesis has been recently reinforced by studies showing that increasing glycolysis supports the virus replication in colon cancer cells and in blood monocytes [28,67]. In monocyes, SARS-CoV2 replication is arrested when pyruvate is only given, this substrate sustaining TCA cycle and oxidative metabolism [67]. This observation provides arguments to consider that hypoxia - the major inducer of the Warburg effect - is an essential condition for promoting SARS-CoV-2 replication. Of note, pneumonia is usually first seen in the basal part of lungs with the lowest ventilation/perfusion ratio [1,32].

In most patients, COVID-19 infection is quickly controlled and recovery is fairly rapid, as the virus is effectively fought by neutrophils and mobile monocytes/macrophages which migrate to the sites of inflammation. This pro-inflammatory innate response (representing the first line of defense of the immune system) with high secretion levels of NO, pro-inflammatory lipids, IL-1, Il-6 and TNF-α, is further followed by dendritic cell (DC) activation. Macrophages and DCs present antigens, a process leading to T cytotoxic lymphocytes and B lymphocytes activation with development of a memory response. Of note, all these activations are supported by the Warburg effect [68]. Thereafter, the anti-inflammatory and restorative healing response occurs, promoted by M2 macrophages and IL-10. As we have seen, AMPK signaling likely plays a key role in the coordination and regulation of the shift towards the anti-inflammatory and repairing response. Indeed, AMPK inhibits PI3K/AKT, and therefore down regulates aerobic glycolysis, FAS and NO synthesis. P53 deregulation (especially promoted by cancer and aging), may also impact the shift towards the anti-inflammatory response. In consequence, the pro-inflammatory response is
triggered and the cytokine storm develops with abnormal IL-1, IL-6 and TNF-8 secretion, promoting in turn the Warburg effect [69].

The uncontrolled cytokine cascade and extensive micro-vessel thrombosis (also supported by the Warburg effect) conspire to promote the occurrence of ARDS, lung destruction and multi-organ failure (MOF). Further studies should clarify the mechanisms altering the shift from M1 to M2 phenotypes. Of note, Zinc is required for ACE2, ACO2 and mitochondrial superoxide dismutase 2 (SOD2) functioning. This latter enzyme reduces oxidative damage created by reactive oxygen ion superoxide, in particular on enzymes such as ACO2, an iron sulfur cluster containing enzyme created by reactive oxygen ion superoxide, in particular on enzymes such as ACO2, an iron sulfur cluster containing enzyme functioning. This latter enzyme reduces oxidative damage caused by reactive oxygen ion superoxide, in particular on enzymes such as ACO2, an iron sulfur cluster containing enzyme sensitivity could be important especially in diabetic and elderly patients [64,70].

In clinical research, the metabolic phenotype of lung and blood macrophages could be studied by markers reflecting either glycolytic function (such as expression of MCT4) or oxidative function (such as expression of MCT1) [71].

Recent in vitro studies performed on blood monocytes argue that the cytokine cascade is the result of the virus replication inside monocytes and macrophages, a replication favored by increasing glucose concentration (and thus by diabetes), and promoting the secretion of inflammatory cytokines by these cells [67]. Blood monocytes could be the cell mediator of the cytokine cascade and/or multi-organ dissemination of the virus. Since only 1% of patients had detectable levels of SARS-CoV-2 in the blood, this suggests that viremia does not underlie the dissemination process [72,73]. Further in vivo studies should specify the sequence of contamination, as the virus enters the body mainly through respiratory tract cells and ileal absorptive enterocytes [29].

It is noteworthy that the Warburg effect may down regulate the adaptive immune response, if we consider that the increase acidity in the extracellular compartment favors inhibition of the cytotoxic immune response in cancer studies [for references, see 10]. In favor, an increased serum lactate dehydrogenase (LDH) level reflects the severity of COVID-19 [74], as well as lymphopenia which means a decreased number of NK cells and cytotoxic T lymphocytes [75,76]. Impairment of immune defense can increase sensitivity to bacterial co-infection, and in this setting overcoming the Warburg Effect has been considered as a key factor to improve tolerance to septic shock, often resulting in MOF and death [77].

As we have seen, atherosclerosis is a pathologic feature frequently observed in elderly patients, a process promoting the Warburg effect in endothelial and smooth muscular cells of the vessels. This pathologic process is favored by many factors (diabetes, obesity, metabolic syndrome, lipids abnormalities) inducing a state of low-grade inflammation, a condition increasing with aging. In parallel, elderly patients have a global reduction in the capacity to cope with infection, which is exemplified by the mortality rate: from 1 to 3% in patients between 50 and 59 years, it increases to 9.8% from 70 to 79 years and it is approximately 14% for around 85 years old [1].

From a therapeutic point of view, counteracting the metabolism sustaining SARS-CoV-2 replication and/or macrophages activation can be essential. AMPK activators such as metformin [78,79], lipic acid [80], resveratrol [81], and ivmecrinit [82] should be tested in vitro and in vivo in a preventive or curative intent. Interestingly, lipic acid also inhibits furin, a convertase involved in increasing SARS-CoV-2 infectivity and virulence [80,83], while ivmecrinit inhibits the virus replication in vitro [84].

Obviously, glucose transporter (GLUT) inhibitors should be tested, as well as phlorizin, a molecule which inhibits glucose absorption by the apical transporters Na+-dependent glucose transporter 1 (SGLT1) of intestinal cells, as showed with the coronavirus transmissible gastroenteritis virus (TGEV) [23]. This molecule also inhibits glucose uptake by the alveolar-airway barrier [85].

### Table 1

| Pathway targeted | Inhibitors |
|------------------|-----------|
| Glycolysis       | Fasentin, Phloretin (GLUT2 inhibitor), Ritonavir (GLUT4 inhibitor), Silybin/Silibinin, STF-31 (GLUT1 inhibitor) Pitoridizin (SGLT1 inhibitor), Mevalonate and cholesterol synthesis, Citrate sodium, Sulforaphane |
| GLUTs            | SGLT2 inhibitor, Citrate sodium, Sulforaphane |
| SGLT2s           | Bis-2-(5-phenylacetamido-1,3,4-thiadiazol-2-yl)ethyl sulfonyl (BPTES) |
| HK2              | Atractylisin, Benserazide, 2-deoxyglucose, Genistein-27, Lonidamine, Resveratrol |
| PKR              | Resveratrol, Apgenin |
| PKR2/PKRFB3      | PFK15 |
| GAPDH            | 3-bromopyruvate (inhibits also HK2, PKG1, IDH) |
| PKM2             | Resveratrol, Apgenin |
| LDH-A            | FX11, Oxamate |
| Inhibiting the Warburg effect by reconnecting TCA cycle | Lipic acid |
| PDH activation   | Dichloroacetate |
| PDK inhibition   | Metformine, Lipic acid, Resveratrol, Ivermecrinit |
| AMPK activator   | Metformine, Lipic acid, Resveratrol, Ivermecrinit |
| Lactate exchanges | MCTs: AZD-3965, Oxamate |
| glutaminolysis   | Benzylerine, GPNAV-9302 |
| ASCT2 (SLC1A5)   | Asasaine, Acacivin, BPTE3, CB-839, DON, Zaprinast |
| GLS1             | IDH305, Glutamidulin, AG-120 (IDH1 inhibitor), AG-221 |
| IDH              | IDH305, Glutamidulin, AG-120 (IDH1 inhibitor), AG-221 |
| Lipid synthesis  | AZT |
| ACLY             | Bempedoe acid, Cucurbitacin B, Hydroxyccitrace |
| FAS inhibition   | TVB-2640, Cerulenin, Epigalocatechin Gallate, Orlistat |
| Mevalonate and cholesterol synthesis | Statins |

ACLY: ATP citrate lyase; AMPK: AMP-activated protein kinase; ASCT2: glutamine transporter 2; BPTE3: Bis-2-(5-phenylacetamido-1,3,4-thiadiazol-2-yl)ethyl sulfide; DON: 6-Diazo-5-oxo-L-norleucine; FAS: fatty acid synthesis; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; GLUTs: glucose transporters; HK2: hexokinase 2; IDH: isocitrate dehydrogenase; LDH-A: lactate dehydrogenase A; PDH: pyruvate dehydrogenase, PDK: pyruvate kinase dehydrogenase; PFK: phosphofructokinase; PFK15: 1-(4-pyridinyl)-3-(2-quinolinyl)-2-propen-1-one; PKG1: phosphoglycerate kinase1; PKM2: pyruvate kinase M 2; 3PO: 3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one, SGLT1: Sodium dependent glucose transporter 1.
Consideration should be given to inhibitors of aerobic glycolysis and its branched pathways, as well as FAS-stabilized membrane replication [86]. The effect of glutamine metabolism inhibitors should be studied in particular with respect to the metabolic vulnerabilities of SARS-CoV-2 replication. In the past, L-asparaginase greatly increased the efficiency of anti-nucleotide agents in acute leukemia. However, targeting one specific pathway may result in modest viral replication inhibition, as the virus can up-regulate or become dependent on alternative pathway(s) to meet bioenergetic or biosynthetic needs. As an example, human fibroblasts infected with HCMV failed to produce virions when starved for glutamine 24 h after infection [87]. Such resistance can be favored by the competition for nutrients occurring at the site of inflammation, a process well-observed in the microenvironment of cancer cells [88]. A (non-exhaustive) list of candidate inhibitors (well-studied in process well-observed in the microenvironment of cancer cells competition for nutrients occurring at the site of inflammation) is presented in Table 1. Animal models like ferret or hamster model of Covid-19 can be useful for testing drugs because in vitro studies are not always confirmed by in vivo studies.

Finally, in the context of the current pandemic and in the perspective of new ones, the exploration of all aspects of the Warburg effect in COVID-19 is certainly fundamental to the discovery of new treatments.

**Authors’ contribution**

Philipp Icard: conception, writing, revision; Hubert Lincet: figure design and revision; Zherui Wu: figure and table design; Antoine Coquereul: literature search and revision; Patricia Forgez: literature search and revision; Marco Alfiano: revision and supervision; Ludovic Fournel: editing and revision.

**Declaration of competing interest**

The authors have no conflict of interest to declare.

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