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# Potassium and Sodium Channels and the Warburg Effect: Biophysical Regulation of Cancer Metabolism

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**Abstract**

Ion channels are progressively emerging as a novel class of membrane proteins expressed in several types of human cancers and regulating the different aspects of cancer cell behavior. The metabolism of cancer cells, usually composed by a variable proportion of respiration, glycolysis, and glutaminolysis, leads to the excessive production of acidic metabolic products. The presence of these acidic metabolites inside the cells results in intracellular acidosis, and hinders survival and proliferation. For this reason, tumor cells activate mechanisms of pH control that produce a constitutive increase in intracellular pH ($pH_i$) that is more acidic than the extracellular pH ($pH_e$). This condition forms a perfect microenvironment for metastatic progression and may be permissive for some of the acquired characteristics of tumors. Recent analyses have revealed complex interconnections between oncogenic activation, ion channels, hypoxia signaling and metabolic pathways that are dysregulated in cancer. Here, we summarize the molecular mechanisms of the Warburg effect and hypoxia and their association. Moreover, we discuss the recent findings concerning the involvement of ion channels in various aspects of the Warburg effect and hypoxia, focusing on the role of $Na^+$ and $K^+$ channels in hypoxic and metabolic reprogramming in cancer.

**Keywords:** ion channels, Warburg effect, cancer



## Introduction

Metabolic alterations represent one of the emerging hallmarks of cancer.[1] During cancerogenesis, neoplastic cells occupy increasingly ''poorer'' microenvironments and to maintain their viability and build new biomass, cancer cells rely strongly on their ability to utilize low-level nutrients present in the microenvironment and/or supplied by a progressively deficient vascularization.[1,2] Thus, it is not surprising that cancer cells reprogram their metabolism during tumor progression. Such altered metabolism not only permits cancer cells to survive under adverse conditions, such as hypoxia, but also enables their proliferation, growth, invasiveness, and metastasis.[2] Such reprogramming can occur as both direct and indirect consequences of oncogenic alterations, including mutations. Conversely, the alterations in intracellular and extracellular metabolites that accompany cancer-associated metabolic reprogramming can have profound effects on gene expression, cellular differentiation, and on the tumor microenvironment itself, thus creating a vicious circle.[2]

Several metabolic changes characterize cancer cells: deregulated glucose uptake and utilization, increased gluconeogenesis and increased glutaminolytic activity, reduced fatty acid oxidation, increased *de novo* fatty acid synthesis, increased glycerol turnover, modified amino acid metabolism, and increased pentose phosphate pathway (PPP) activity.[2,3] One of the metabolic alterations most commonly displayed by many, if not all, cancer types, is an increased rate of intracellular glucose import and a higher rate of glycolysis associated with reduced pyruvate oxidation and increased lactic acid production, the so-called Warburg effect.[4] In turn, the increased lactate production contributes to extracellular acidification, another characteristic of malignant transformation. The enhanced glycolysis in cancer cells was initially traced back to insufficient cellular respiration as causative of cancer (Warburg's own hypothesis). Subsequently, however, it was shown that the Warburg effect is not a primary cause of malignancy,[5] but a downstream condition. Nevertheless, such an metabolic alteration has been exploited clinically for diagnostic and prognostic purposes. For example, in positron emission tomography, imaging of the uptake of a radioactive fluorine-labeled glucose analog, $^{18}$F-fluorodeoxyglucose, is used for cancer diagnosis and staging, as well as for monitoring response to treatment.[6]

The cause(s) of the upregulation of glycolysis in cancer has not been conclusively elucidated, and several hypotheses have been proposed, including microenvironmental adaptation,[7] conferral of biosynthetic advantages[8] and the presence

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of hypoxic conditions inside malignant and premalignant lesions.[9] Indeed, hypoxia is an intrinsic characteristic of growing tumors, to which cancer cells adapt by increasing angiogenesis and which, in turn, may cause genetic instability and hence accumulation of further malignancy-driving mutations.[10]

A novel hallmark of cancer cells is the dysregulation of their ionic activity, involving ion channels and transporters (ICTs), transmembrane proteins that are becoming important players in cancer.[11–13] Indeed, the expression and activity of different ion channel types mark and regulate specific stages of cancer progression and their contribution to the neoplastic phenotype ranges from control of cell proliferation and apoptosis, to regulation of invasiveness.[11–13] While substantial work has been done on a range of tumor cell lines, recent evidence has shown that blocking channel activity can impair tumor growth and metastasis also *in vivo*.[14] Consequently, altered ICT expression can be exploited for diagnostic purposes and/or for targeting traceable or cytotoxic compounds to specific neoplastic tissues.[15]

The aim of the present review is to summarize current knowledge about the possible role of ICTs in the Warburg effect. We first describe (i) the involvement of hypoxia in the Warburg effect and (ii) the main pathophysiological characteristics of ICTs in cancer, focusing on $Na^+$ and $K^+$ channels. Finally, we evaluate a possible converging hypothesis involving the Warburg effect, hypoxia, and $Na^+$ and $K^+$ channels, and we discuss the emerging strategies to target ICTs in tumor treatment.

## The Warburg Effect

### *Overview of the general mechanisms of increased aerobic glycolysis in cancer*

Following the observation of a decrease in oxygen uptake caused by glucose in cancer cells, the so-called Crabtree effect, Otto Warburg showed that malignant transformation is associated with an increased rate of cellular glucose import and that a higher glycolysis rate is associated with reduced pyruvate oxidation and increased lactic acid production.[4,16,17] In normal conditions, glycolysis occurs when the oxygen concentration is low, but in cancer it also occurs in the presence of adequate oxygenation. Hence, cancer cells are characterized by the so-called aerobic glycolysis,[4,17,18] this metabolic shift being maintained throughout tumor progression,

Working under the assumption that the major use of glucose in cancer cells was to generate adenosine triphosphate (ATP), Warburg and others misinterpreted this phenomenon, attributing it to the ''irreversible injuring of respiration.''[17]

In normal cells, influx of glucose into normal cells is not driven by the immediate bioenergetic needs of a cell but is strictly rationed as a result of extracellular stimuli. Furthermore, in case of growth factor deprivation, cell survival can be restored by upregulation of the glucose transporter 1 (GLUT-1) on the plasma membrane as well as hexokinase (HK).[19] On the contrary, cancer cells accumulate oncogenic alterations that make their metabolic requirements to be substantially independent of external stimuli.[4] Such changes involve phosphoinositide 3 kinase (PI3K), its negative regulators phosphatase and tensin homolog (PTEN) and INPP4B, and activating mutations and gene amplifications in a variety of upstream receptor tyrosine kinases.[1,20] PI3K/Akt signaling acts as a master regulator of glucose uptake, promoting both the mRNA expression of *GLUT-1* and translocation of the protein from endomembranes to cell surface.[21,22] In addition, Akt potentiates the activities of HK and phosphofructokinase (PFK), which catalyze the key irreversible step of glycolysis.[23,24] Overall, multiple growth factor signaling pathways aberrantly activated in cancer can facilitate the utilization of glucose by cancer cells[7] (Table 1). When a tumor cell is ''quiescent'', similar to normal cells, glucose is preferentially utilized for generation of acetyl-CoA, which is then oxidized in the tricarboxylic (TCA) cycle in mitochondria. In turn, this produces NADH and FADH2 and contributes to the generation of the electrochemical gradient that fuels ATP production. Still, the ''carbon economy'' of a proliferating normal cell differs dramatically from that of a quiescent cancer cell.[8] The major use of reduced carbon in proliferating cells is for the biosynthesis of a diverse array of biomolecules. To accomplish this, nutrients must be transformed into diverse pools of structural intermediates. In these reactions, the donor of reducing equivalents is NADPH. Hence, a proliferating cell must allocate a portion of its carbon substrates to be used in NADPH production instead of NADH to support mitochondrial electron transport. The reprogramming of carbon metabolism by proliferating cancer cells provides part of the explanation for Warburg's original observations concerning tumor metabolism. Rather than being an adaptation to a defect in respiration, the Warburg effect is a regulated metabolic state and may, in fact, be beneficial during a time of increased biosynthetic demand.[4]

It was recently shown that by converting excess pyruvate to lactate, proliferating cells prevent accumulation of cytosolic NADH and reduce ATP production. In turn, this promotes continued cytosolic glucose metabolism free from feedback repression by excess ATP generated in mitochondria.[3] It is worth recalling that a number of glycolytic intermediates can be diverted into branching pathways, generating diverse biosynthetic precursors. First, the PPP, in which glucose-6-phosphate becomes partially oxidized to generate NADPH and ribose-5-phosphate, a structural component of nucleotides. Utilization of PPP is frequently elevated in tumorigenesis, and both oncogenes and tumor suppressors regulate PPP activity, for example, Ras activates it[31] while p53 is inhibitory.[32] Second, fructose-6-phosphate, which follows glucose-6-phosphate in the glycolytic pathway, can also leave glycolysis and become utilized as a substrate for hexosamine biosynthesis. The hexosamine pathway provides substrates for cellular glycosylation reactions (as well as heparan sulfate and hyaluronic acid biosynthesis), potentiates receptor-mediated signaling, and regulates the stability of select proteins, including the transcription factor, c-myc.[33–35] Third, dihydroxyacetone phosphate, is utilized in the biosynthesis of diverse phospholipids, a major structural component of cellular membranes. Fourth, 3-phosphoglycerate is used as a precursor for biosynthesis of serine and glycine, and as a means to generate methyl donor groups and NADPH.[36,37] Serine has a unique metabolic role as a major substrate for the so-called one-carbon or folate cycle, which in turn contributes to the biosynthesis of purines, thymidine, as well as in the production of S-adenosylmethionine, a principal substrate for cellular methylation reactions.[38] In addition, stepwise oxidation of one-carbon tetrahydrofolate species was recently shown to produce up to 50% of all cellular NADPH.[39] Thus, one-carbon pathway metabolites contribute to a number of cellular biosynthetic and

Table 1. Warburg Regulatory Factors and Their Role in Cancer

| *Warburg regulatory factors* | *Function* | *Role in cancer cell* | *Glycolytic regulation* | *Regulation by $Na^+$ and $K^+$ channels* |
|---|---|---|---|---|
| Glut-1 | Membrane transporter. | GLUT1 expression is upregulated in a wide variety of tumors and is likely to be an essential process in tumor progression.[7] | Glucose transport into the cells. It is also capable of transporting mannose, galactose, glucosamine, and reduced ascorbate.[7] | Nonmetastatic colorectal cancer patients showed a correlation between the expression of hERG1 and GLUT1. Particularly the presence of hERG1, associated with the lack of GLUT1 expression, was associated with a worse prognosis for patients with stage I/II colorectal cancer.[25] |
| Hexokinase | Glycolytic enzyme. Converts glucose to glucose-6-phosphate. | Expression is increased in several cancers. | Rate-limiting enzyme and first to phosphorylate glucose once inside the cell. Enables more glucose entry into the cell via its concentration gradient. | HK1 forms coimmunoprecipitatable complexes with both VDAC and GLT-1, it does not physically link GLT-1 to mitochondrial proteins. However, the interaction of HK1 with VDAC supports GLT-1-mediated transport activity.[26] |
| PFK, Phosphofructokinase | Glycolytic enzyme. Converts fructose-6-phosphate to fructose-1,6- bisphosphate. | PFK activity is elevated in cancer cell lines and primary tumors *in situ* particularly in response to proliferating signals originating from activation of *ras* and *src* or HIF-1$\alpha$. | It is critical in determining the rate of glycolytic flux. | |
| PI3K/AKT | Oncogene signaling pathway. | Activated in cancer cells by several mechanisms, including *PTEN* mutations. | Increases expression and plasma membrane clustering of glucose transporters. Increases expression and activity of hexokinase II. Promotes association of hexokinase with mitochondria. Increases expression of phosphofructokinase. | 17$\beta$-estradiol attenuates LPS-induced ALI not only by repressing inflammation but also by reducing pulmonary edema via elevation of $\alpha$-ENaC expression and membrane abundance. These effects were mediated, at least partially, via activation of the PI3K/Akt/SGK1 signaling pathway.[27] |
| cMYC and beta catenin | Transcription factors. | Cancer cells take advantage of *Myc*'s broad reach to reprogram and augment the most critical processes for survival, particularly metabolism. | Induce expression of glycolytic genes such as hexokinase II, enolase, lactate dehydrogenase, phosphofructokinase, and MCT1. | Mice with a heterozygous $APC^{Min}$ mutation develop multiple intestinal neoplasia (Min) leading to premature death. Early in colorectal carcinogenesis, $APC^{Min/+}$ mice show enhanced Akt-mammalian target of rapamycin (mTOR) signaling, which is paralleled by upregulation of oncogenic $K^+$ channels.[28] |
| P53 | Tumor suppressor gene. Guardian of genome. | Mutated in several cancers. | Through loss of TIGAR and SCO2. | The blockade of BK channels results in tumor cell apoptosis and cycle arrest at $G_1$ phase, and the transduction pathway underlying the antiproliferative effects is linked to the increased expression of apoptotic protein p53 and the decreased expression of its chaperone proteins hsp.[29] |
| PK Pyruvate kinase | Glycolytic enzyme. Catalyzes the transphosphorylation of phosphoenolpyruvate into pyruvate and ATP. | The M2 isoform of pyruvate kinase (PKM2) supports anabolic metabolism and is expressed both in cancer and normal tissue. The expression of PKM2 is increased in the majority of cancer cells. | Rate-limiting terminal enzyme of glycolysis. | Pyruvate kinase interacts with Kir6.2 subunits in rat ventricles. Direct evidence for the concept that key enzymes involved in glycolytic ATP production are part of a multisubunit $K_{ATP}$ channel protein complex.[30] |

regulatory processes. Finally, methylene tetrahydrofolate dehydrogenase 2, a component of the mitochondrial arm of the one-carbon pathway, was found to be among the top three most frequently overexpressed metabolic enzymes in cancer, suggesting that alterations in this pathway may be universally selected for tumorigenesis.[40]

In conclusion, glycolytic intermediates leave glycolysis to take part in diverse biosynthetic reactions. Consequently, the rate-limiting enzymes within branching pathways of glycolysis are frequently upregulated in tumors. Thus, proliferating cells have evolved a novel mechanism to regulate the last step of glycolysis, balancing its biosynthetic outputs with providing pyruvate that supports the TCA cycle. This step is regulated by pyruvate kinase (PK), an enzyme that converts phosphoenolpyruvate to, the final product of glycolysis, pyruvate. With the exception of liver and kidney, which express tissue-specific PK isoforms (PKL and PKR, respectively), most tissues express the PKM (muscle) form of PK. The latter exists as two splice variants.[41] While PKM1 is more efficient at producing pyruvate, the majority of proliferating cells and essentially all cancer cells express primarily the PKM2 variant.[42] Importantly, unlike PKM1, the activity of PKM2 is highly regulated, inhibited by tyrosine and activated by serine phosphorylation.[43–45] In such a manner, growth factor signaling inhibits PKM2, leading to accumulation of glycolytic intermediates until the growing cell's need for a catabolizable pool of free serine is saturated. The emerging evidence favors the hypothesis that glycolysis is utilized by proliferating cells as a versatile production line that generates metabolic intermediates for numerous biosynthetic processes. Any excess, nonutilized glycolytic flux is preferentially converted to lactate to help preserve a sufficient pool of $NAD^+$ to sustain glycolysis and avoid flooding the mitochondria with NADH that would suppress the TCA cycle.[46]

A variety of oncogenes have been shown to contribute to the metabolic adaptations of proliferating cells, as outlined above. For instance, c-myc coordinates increase in the expression of (i) pyruvate dehydrogenase kinase isozyme 1 (PDK1), (ii) lactate dehydrogenase A (LDHA), an enzyme that catalyzes the reductive conversion of pyruvate to lactate, and (iii) monocarboxylate transporter (MCT1), facilitating the efflux of lactate into the extracellular space.[47] In addition to c-myc, b-catenin/T cell factor signaling has also been shown to upregulate MCT1 and PDK1 transcription.[48] Finally, hypoxia or different oncogenic stress can also trigger coordinated transcriptional upregulation of LDHA and PDK1.[49,50] Even with these adaptations, however, proliferating cells often accumulate electron transport flux that exceeds the capacity of the ATP synthase, resulting in the formation of excess reactive oxygen species (ROS). In fact, the damaging consequences of such overproduction of ROS may underlie the phenomenon of oncogene-induced cellular senescence.

### *Overview of molecular mechanism of hypoxia and of hypoxia-induced metabolic reprogramming in cancer cells*

Hypoxia, reduction in the normal level of tissue oxygen tension ($pO_2$) to less than 15 mmHg, is a common characteristic of locally advanced solid tumors. This occurs as a consequence of the uncontrolled proliferation of cancer cells that quickly consume nutrients and oxygen supply from the vasculature. Cancer cells are able to sense changes in oxygen tension and to develop adaptive changes, enabling survival and even prolonging proliferation. These adaptive processes contribute to the development of the malignant phenotype and limit therapeutic responses.[51]

The adaptation to reduced $O_2$ tension is characterized by different hypoxia-stimulated biological mechanisms driven by the hypoxia-inducible factor 1 (HIF-1) (Table 2). HIF-1, identified in 1992 by Semenza and Wang, is a mediator of the primary transcriptional adaption to hypoxic stress in cancer, upregulating the expression of erythropoietin.[52] HIF-1 is a heterodimer comprising two subunits, the cytoplasmic hypoxic response factor HIF-1$\alpha$ and the aryl hydrocarbon receptor nuclear translocator (ARNT), also known as HIF-1$\beta$.[53] Whereas HIF-1$\beta$ is constitutively expressed independently of $pO_2$ tension, HIF-1$\alpha$ is responsive to $O_2$ levels. Under normoxic conditions (21% $O_2$), HIF-1$\alpha$ is hydroxylated by the prolyl hydroxylase domain protein (PHD), which promotes binding to the von Hippel–Lindau (VHL) protein.[54] This interaction leads to ubiquitylation and rapid degradation of HIF-1$\alpha$. Since the activity of PHD requires $O_2$, it cannot modify HIF-1$\alpha$ under hypoxic conditions. In cells exposed to reduced $O_2$ levels, stable HIF-1$\alpha$ subunits translocate to the nucleus where they heterodimerize with HIF-1$\beta$ subunits, leading to an active HIF-1 protein that binds to specific hypoxia-response elements (HREs) and regulates the transcriptional activity of numerous target genes.[55]

Since its discovery, HIF-1 has been shown to be associated with several biological pathways such as the induction of neovascularization.[56] HIF-1 activates the transcription of vascular endothelial growth factor (VEGF) in cancer cells and of its receptor VEGR1 in endothelial cells. HIF-1 also induces expression of growth factors that stimulate proliferation and promote proteolysis and factors that stimulate cellular migration.[51,57] Moreover, the constitutive expression of HIF-1, resulting from genetic alterations, could lead to tumor invasion.[58] On the contrary, HIF-1 can also regulate proapoptotic genes, causing autophagy or cell death. In the latter, HIF has been linked to p53, although both interactive and competitive mechanisms have been described.[59] Further studies relating necrotic regions of tumors to blood vessel disposition suggested that necrosis was, at least in part, driven by hypoxia. This dual effect of hypoxia in solid tumors has been described as a direct consequence of the oxygen gradient. Besides PHD-mediated regulation, HIF-1 action is inhibited by another oxygen sensor, the asparaginyl hydroxylase factor inhibiting HIF-1 (FIH).[60] The catalytic profiles of these two hydroxylases are different in the oxygen sensing pathway.[61] FIH hydroxylates an asparagine residue (N803) in the carboxy-terminal transactivation domain of HIF-1$\alpha$. In the peripheral tumor area with mild hypoxia, although PHD activity is arrested and HIF-1$\alpha$ could heterodimerize with HIF-1$\beta$, FIH is still active for its higher affinity to $O_2$ than PHD and, in these conditions, the inhibition of HIF-1$\alpha$ is still maintained in the carboxy-terminal transactivation domain by FIH. In highly hypoxic tumor regions, the catalytic activities of both hydroxylases are inhibited and HIF-1$\alpha$ attains full transcriptional activity, leading to apoptotic and necrotic processes.[61]

There are several other factors, especially various oncogenic pathways that can increase HIF-1$\alpha$ protein levels,

Table 2. Hypoxia Regulatory Factors and Their Role in Cancer

| *Hypoxia regulatory factors* | *Function* | *Role in cancer cell* | *Signaling pathways* | *Regulation by $Na^+$ and $K^+$ channels* |
|---|---|---|---|---|
| HIF-1 | Transcription factor. | Regulates the cellular and homeostatic response to hypoxia.[52,53] | Heterodimerization of HIF-1$\alpha$ and HIF-1$\beta$ subunits.[53–55] | |
| HIF-1$\alpha$ | Alpha subunit of transcription factor hypoxia-inducible factor-1. | Stabilized in cancer even under normoxic conditions.<br>Activates the transcription of many genes, including those involved in cancer cell proliferation (e.g., TGF-$\alpha$), angiogenesis (e.g., VEGF), erythropoiesis, apoptosis (e.g., BNip3 and p53), stress-response pathways, cell adhesion, migration, and pH regulation (CAIX).[51,56–59]<br>Induces glucose consumption and lactate production, upregulating glucose transporters, glycolytic enzymes, LDH-A, and MCT4, and inhibiting PDH through activation of PDK1.[66–72] | Oxygen sensors: the prolyl hydroxylase domain (PHD) proteins and the asparaginyl hydroxylase factor inhibiting HIF-1 (FIH).[60,61]<br>Phosphorylation by MAPK.[62]<br>Oncogenic signaling pathways involving SRC, RAS, Akt, PI3K, and mTOR, PTEN or p53 loss.[61,63–65]<br>Ligands of tyrosine kinase receptors (e.g., EGFR and HER2).<br>Interaction with transcription factors such as AP-1, ETS, and the cyclic AMP-response element-binding protein (CREB).[66–68]<br>CAIX expression and mutations in VHL.[64] | $K_v$10.1 expression increases basal expression levels of HIF-1$\alpha$ and increases response of cells to mild hypoxia. This leads to VEGF secretion and neovascularization.[73]<br>$K_v$11.1 (also named hERG1) couples with $\beta$1 integrin and regulates a signaling pathway that sustains VEGF-A secretion, angiogenesis, and progression in colorectal cancer. In this pathway, $K_v$11.1 recruits and activates PI3K and Akt. This in turn increases HIF-dependent transcription of VEGF-A and other tumor progression genes.[74] |
| ARNT (also known as HIF-1$\beta$) | Binds to ligand-bound aryl hydrocarbon receptor and aids in the movement of this complex to the nucleus. | Cofactor for transcriptional regulation by hypoxia-inducible factor 1.[53] | Constitutively expressed in normoxic conditions. | |

AMP, adenosine monophosphate; AP-1, activator protein 1; ARNT, aryl hydrocarbon receptor nuclear translocator; BNip3, BCL2 interacting protein 3; CAIX, carbonic anhydrase-9; EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor 2; HIF-1, hypoxia-inducible factor 1; $K_v$, voltage-gated potassium channel; LDHA, lactate dehydrogenase A; MAPK, mitogen-activated protein kinases; MCT4, monocarboxylate transporter 4; PDH, pyruvate dehydrogenase; PDK1, pyruvate dehydrogenase kinase 1; PI3K, phosphoinositide 3 kinase; SRC, proto-oncogene tyrosine-protein kinase; VHL, von Hippel–Lindau tumor suppressor; VEGF, vascular endothelial growth factor.



independent of hypoxia.[75] For example, *HRAS-V12* leads to the accumulation of HIF-1$\alpha$ in both normoxic and hypoxic conditions.[76] Increased HIF-1 targets are associated with pathways of Ras-MAPK[62] and PI3K-Akt.[63–65] Direct activation of PI3K itself or the downstream kinase Akt, or loss of the inhibitory PTEN tumor suppressor, leads to increased HIF-1$\alpha$ levels and activity. It has also been demonstrated that Akt increases HIF-1$\alpha$ translation by both mechanistic target of rapamycin (mTOR)-dependent and mTOR-independent mechanisms.[75]

Recently, attention has been focused onto the concept of ''intermittent hypoxia.'' In solid tumors, intermittent hypoxia associated with irregular blood supply can cause the switching of hypoxia and reoxygenation phases. Compared with those induced by chronic hypoxia, this exerts different effects on HIF-1 stabilization and HIF-1 target gene transcription.[77] *In vitro* and *in vivo* studies have shown that intermittent hypoxia affects both tumor and endothelial cells and the interplay between them, resulting in rapidly growing tumors and the development of new vascular networks. Ultimately, intermittent oxygen exposure has been also linked to an accelerated tumor progression, metastasis, and resistance to therapies.[69]

HIF-1 also has an important role in driving aerobic glycolysis to meet the biosynthetic demand and to prevent cells from hypoxic stress damage.[66] The mechanisms underlying the hypoxia-induced metabolic reprogramming in cancer cells are well documented. Low $O_2$ levels increase the need for glucose and upregulate GLUT-1 and GLUT-3 expression. Simultaneously, lactate production is increased enhancing the activity of glycolytic enzymes, including HK1, HK2, PKM2, and LDHA.[66] There is also a feedback mechanism

through which glycolytic enzymes (e.g., PKM2) can regulate HIF-1α activity by stimulating its binding to HRE.[67] During hypoxic conditions, HIF-1α activates PDK1, which inhibits PDH, avoiding the conversion of pyruvate in acetyl-CoA. In these conditions, HIF-dependent activation of PDK1 prevents ATP synthesis and also reduces ROS production protecting the cells from hypoxic stress damage.[68]

Hypoxia also induces changes in glutamine metabolism: the HIF-1α-dependent amplification of the *MYC* oncogene upregulates glutamine-metabolizing enzymes such as glutaminase; hypoxic cells with high MYC levels show increased oxidative metabolism of glutamine.[70] The HIF-1 signaling pathway also leads to the upregulated expression of MCT and $Na^+/H^+$ exchanger.[71] The extrusion of lactate and acid, with pH reduction, is a typical feature of hypoxic cells. Hypoxia contributes to the acid tumor microenvironment, which gives the tumor a growth advantage, also upregulating carbonic anhydrase-9 (CAIX), which exerts a feedback inhibition on VHL protein, favoring the maintenance of high level of stable HIF-1α.[72]

Acidification of $pH_e$ has been associated with nucleolar sequestration of VHL, preventing it from targeting HIF-1α for degradation. This suggests that the acidic tumor microenvironment may contribute to the metabolic shift, favoring the maintenance of high HIF-1α levels. It is also evident that acidification induced cellular mechanisms that can upregulate the expression of transporters, such as NHE1 and NBCn1. Moreover, hypoxia, through HIF-1α, promotes the expression of several plasma membrane-located transporters, exchangers, pumps, and ectoenzymes. Since altered $pH_i$ and $pH_e$ in tumors may modulate cancer development through effects on ion channels, we speculate that HIF-1α could affect also ion channels, in particular pH-sensitive $K^+$ channels. Indeed, an important example is the family of two-pore $K^+$ channels, many members of which are highly pH sensitive in the relevant range. Among these, the subfamily, including TASK-2, TALK-1, and TALK-2, is activated by extracellular alkalinization (pK½ for TASK-2 is 8.0) and hence would be expected to be inhibited in the acidic tumor microenvironment. TASK-2 is additionally opened by intracellular alkalinization, and yet, this cannot overcome the inhibitory effect of acidic $pH_e$. The regulation of TASK channels by tumor $pH_e$ is of particular interest because TASK channels, at least in some cell types, are required for apoptosis, due to their role in apoptotic volume decrease. This suggests that acidic tumor $pH_e$ may contribute to death avoidance in cancer cells through inhibition of two-pore $K^+$ channels. Complicating the picture, these channels are also implicated in cell cycle progression, which would thus be counteracted by their acid-mediated inhibition. In particular, TASK-3, which is inhibited by extracellular acidification, is overexpressed in ovarian cancer, and TASK-3 blockers caused a significant reduction in cell proliferation and an increase in apoptosis in SKOV-3 and OVCAR-3 ovarian cancer cells.[78]

## Ion Channels in Cancer: Focus on Sodium ($Na^+$) and Potassium ($K^+$) Channels

Ion channels are integral membrane proteins that play a key role in a variety of cell functions. Moreover, they have been emerging as a novel class of membrane proteins expressed in several types of human cancers.[79] In cells, $K^+$ channels are involved in cell's resting membrane potential,[80] volume control,[81] cell cycle progression,[82] repolarization,[83] and cell proliferation.[84] Here, we summarize the current findings concerning the role of ion channels in cancer, focusing on $Na^+$ and $K^+$ channels. Readers interested in a complete description of $Na^+$ and $K^+$ channel members and functions can refer to the following reviews.[11–15]

Voltage-activated sodium channels (VGSCs) drive $Na^+$ influx in excitable cells and are involved in the action potential generation and propagation.[85] VGSC expression has been found also in ''nonexcitable'' cells such as lymphocytes, osteoblasts, and endothelial cells.[85] It was demonstrated that VGSCs are expressed in breast[86,87] prostate,[88,89] lung,[90,91] uterine cervix,[92] and colorectal cancer[93] and mesothelioma.[94] In particular, it was demonstrated the VSGC Nav1.5 is selectively upregulated in metastatic breast cancer *in vivo* and *in vitro* and that VGSC activity is involved in metastasis.[86,93,95] Nav1.6 and/or Nav1.7 α-subunits have been described in melanoma and lung and breast cancer.[95] Nav1.9 expression is correlated with metastatic potential and its activity increases cell migration in prostate cancer,[88] and invasiveness in nonsmall-cell lung cancers.[96]

Among potassium channels, voltage-gated potassium channel ($K_v$) has been reviewed in several publications.[97,98]

Expression of *ether-a gò-gò* 1 (Eag1, also known as Kv10.1 or KCNH1) has been demonstrated in cell lines of different histogenesis (neuroblastoma, breast, melanoma, colon, lung, uterine cervix, and ovary)[99] and it was shown that it is expressed in human tumors, while being absent in the corresponding normal tissues. $K_v$10.1 is expressed in esophageal squamous-cell carcinoma compared with the corresponding normal tissue and the protein correlates with depth of invasion.[100]

$K_v$11.1 (hERG1) is overexpressed in human primary cancers such as endometrial,[101] colorectal,[102] esophageal,[103] pancreatic,[104] ovarian,[105] and brain cancers,[106] as well as leukemias.[107,108] It has been shown that the protein is highly expressed in adenocarcinomas with respect to hyperplastic lesions of endometrium[101] and colon.[102] In the latter, a correlation between invasive phenotype and high $K_v$11.1 levels of expression has been shown.[102] Moreover, $K_v$11.1 positivity and GLUT-1 negativity represent an independent negative prognostic factor in TNM-I and TNM-II colorectal adenocarcinomas,[25] identifying patients whose survival is worse than stage TNM-III patients.[109] $K_v$11.1 channel shows a significant association with malignant progression toward adenocarcinoma[103]; recently, the importance of $K_v$11.1 as a biomarker has been demonstrated.[110] $K_v$11.1 channels are overexpressed also in gastric adenocarcinomas[111] and in lung cancer cell lines where they regulate cell proliferation.[112]

$K_v$1.3 is deregulated during prostate cancer progression, in particular it is expressed in early stages, and downregulated in high-grade cancers,[113,114] and its expression is significantly increased in higher stage breast cancer.[115] $K_v$3.4 is highly expressed in precancerous lesions and in oral squamous cell carcinoma compared with normal oral mucosa.[116]

$Ca^{2+}$-activated $K^+$ channels ($K_{Ca}$'s) are also involved in cancer. For example, functional $K_{Ca}$3.1 is expressed at high levels on the plasma membrane of tumor cells of different histogenesis: prostate adenocarcinoma,[117] pancreatic[118] and breast cancer,[119] gliomas,[120] melanoma,[121] and leukemias.[122] $K_{Ca}$1.1 is amplified in late-stage prostate cancer.[123] $K_{ir}$3.1 is upregulated in pancreatic ductal adenocarcinoma (PDAC).[124]

$K_{2P}$ family, in particular, $K_{2P}9.1$ and $K_{2P}5.1$ are expressed in breast cancer[125]; $K_{2P}2.1$ regulates cell proliferation of prostate cancer.[126]

In the inner membrane, there are ion channels (in particular, $K^+$ channels) that affect mitochondrial metabolic activity, ROS synthesis, and other functions that are modified in tumor cells. For these reasons, these channels are promising targets for oncological therapies. In this case, ion channel blockers are natural candidates for possible therapies. In fact, a positive relationship was found between the mito$K_{ATP}$ channel expression and the proliferation in glioma cells. In contrast, in gastric cancer, impaired mito$K_{ATP}$ channel opening is implicated in curcumin-induced apoptosis. Inhibitors of the Kv1.3 channel (Psora-4, PAP-1, and clofazimine) are able to cause apoptosis by directly targeting mitoKv1.3. The mitoI$K_{Ca}$ channel can be selectively inhibited by low concentrations of clotrimazole and TRAM-34. This drug has been reported to synergistically increase the sensitivity of melanoma cells to the death receptor ligand, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), via its action on the mitoI$K_{Ca}$ channel. The reduction of TASK-3 expression in WM35 melanoma cells compromised mitochondrial function and cell survival. Reduced TASK-3 expression in WM35 and A2058 melanoma cells decreased the viability, reduced the total DNA content, changed the cell morphology, and promoted apoptosis.[127]

## Relationships Between $Na^+$, $K^+$ Channels and the Warburg Effect: A Unifying Landscape

Whereas the role of $K^+$ and $Na^+$ channels in metabolism of cancer is still under study, more evidence exists for noncancer cells (Table 1). For example, in epithelial cells, $Na^+$ -gradients are typically used to cotransport glucose across the cell membrane.[128] Also in cancer cells, glucose transport is facilitated by sodium-dependent glucose transporters (SGLT). SGLT2 was found functionally expressed in pancreatic and prostate adenocarcinomas, and its inhibition decreased glucose uptake and reduced tumor growth in a pancreatic cancer xenograft.[129] In astrocytes, lactate release is stimulated by $K^+$ channels or depolarization,[130] inwardly rectifying $K^+$ channels 4.1 ($K_{ir}4.1$) involved in glutamate uptake,[131] and the voltage-gated $K^+$ channel $K_v1.3$ regulates GLUT-4 protein translocation at the plasma membrane.[132] As concerning cancer, immunohistochemistry on surgical samples of nonmetastatic colorectal cancer patients showed a correlation between the expression of $K_v11.1$ and GLUT-1, as described above.[25,109]

Dichloroacetate (DCA) has been tested as a potential metabolic target therapy for cancer,[133] and its effect has been associated with changes in expression of $K_v$ 4.1. DCA inhibits PDK, shifts the metabolism from glycolysis to glucose oxidation, and suppresses tumor growth, *in vitro* and *in vivo*.[134] The DCA-mediated cell growth inhibition undergoes increased $K_v1.5$ channel expression in breast cancer cell lines.[135]

It has been demonstrated that for their ATP production, gliomas depend disproportionately on glycolytic mechanisms versus oxidative phosphorylation. This event leads to increased proton-extruding proteins ($Na^+/H^+$ antiporter NHE1 and $H^+$-ATPase) and, finally, to decreased extracellular pH. Spheroids obtained with glioma cells showed that changes in external pH, as a consequence of Warburg effect, are sensed by $H^+$-sensitive $K^+$ channels. These ion channels translate these changes by modulating membrane voltage ($V_m$) and glioma cell proliferation and tumorigenesis.[136]

## Current Knowledge About the Relationship Between Hypoxia and $Na^+$ and $K^+$ Channels

In 1988, Lopez-Barneo gave the first description of hypoxia-regulated ion channel, demonstrating that rabbit carotid body glomus cells express an $O_2$-sensitive $K^+$ channel.[137] Since this discovery, numerous studies have been carried out showing that several ion channels, belonging to different families, are $O_2$ sensitive and display changes in their activity with acute hypoxia and their expression with prolonged hypoxic challenge.[138] The first response of many cell types to hypoxia is characterized by modifications in internal $Ca^{2+}$ concentration ($[Ca^{2+}]_i$). Many evidence exist about the involvement of different $K^+$ channel subtypes in this mechanism: the hypoxia-induced membrane depolarization is mediated by the inhibition of the $O_2$-sensitive $K^+$ channels, including $K_v11.1$, that activate voltage-gated $Ca^{2+}$ channels, causing an increase in $[Ca^{2+}]_i$.[138,139]

Evidence of hypoxia-regulated ion channels exist also in cancer. A study carried out on the human melanoma cell line IGR1, reported that $K_{Ca}$ channel activity is enhanced by chronic hypoxia, and hypoxia mimetics, and that the HIF sensory system is responsible for this process.[140] Furthermore, cell proliferation is enhanced under hypoxic condition, and the use of $K_{Ca}$ channel blockers slows down cell growth.[141]

The role of $K^+$ channels as regulators of hypoxia-mediated responses in cancer has been also investigated (Table 2). HIF-1-mediated responses could be enhanced by members of the *ether-à-go-go* family. Their expression has been increasingly linked to oncogenesis and tumor progression. The expression of HIF-1$\alpha$ has been correlated to $K_v10.1$ level in breast cancer tissues.[142] These data are in agreement with those obtained in cell lines, showing that $K_v10.1$ expression increases HIF-1$\alpha$ levels and thus VEGF secretion, inducing tumor vascularization.[73,97] Resistance to hypoxic condition could also be enhanced by the overexpression of the $K_{2P}$ channel KCNK9 in human breast and small-cell lung tumors.[143] Recently, also $K_v11.1$ expression has been related to enhanced HIF(s) transcriptional activation, sustaining angiogenesis and progression in colorectal cancer. The $K_v11.1$-dependent regulation of HIF-1$\alpha$, through the PI3K/Akt pathway, is triggered in normoxia, especially after colorectal cancer cells have experienced a hypoxic stage. Moreover, blocking $K_v11.1$ switches this pathway off *in vitro* and *in vivo*, by inhibiting cell growth, angiogenesis, and metastatic spread.[74]

Recently, the role of another $K_{2P}$ channel, TASK-1 (or KCNK3), was investigated in a subset of nonsmall-cell lung cancers. TASK-1 expression influenced cell proliferation and the channel current was inhibited in hypoxia. Moreover, $Na^+$-coupled nutrient transport across the cell membrane is functionally coupled to the efflux of $K^+$ via $K^+$ channels, and thus, TASK-1 may potentially influence $Na^+$-coupled nutrient transport.[144] In Table 3, a list of oxygen-sensitive $K^+$ and $Na^+$ channels in cancer is reported.

## Antibodies as Tool to Target Macromolecular Complexes Involved in Hypoxia Signaling

As reported above, it is well known the importance of ion channels in a variety of cancer aspects. In particular, their role

Table 3. List of Oxygen-Sensitive $K^+$ and $Na^+$ Channels

| *Channel name* | *Tumor type* | *Hypoxia effect* |
|---|---|---|
| $K_{Ca}$ Channels | Melanoma (IGR1 cells) | Cell proliferation is enhanced[140,141] |
| $K_{Ca}1.1$ | Glioma (LN 2 cells) | Acute hypoxia activates $K_{Ca}1.1$ channels residing in the mitochondrial membrane, while no effect was observed on plasma membrane $K_{Ca}1.1$ channels[145] |
| $K_v3.1$, $K_v3.4$ | Alveolar adenocarcinoma (A549 cells), breast (MDA-MB-231 cells), and colon (HT-29 cells) | Cell density is incremented by HIF-1$\alpha$, and expression of Kv3.1 and Kv3.4 showed the same pattern with HIF-1$\alpha$[146] |
| $K_v10.1$ | Breast (tissues) | Correlation of $K_v10.1$ expression with HIF-1$\alpha$ expression[142] |
| | breast (MDA MB 231 cells) | $K_v10.1$ expression increases HIF-1$\alpha$ levels and thus VEGF secretion, inducing tumor vascularization[73] |
| $K_v11.1$ | Colorectal cancer (HCT116 cells) | $K_v11.1$ expression is related to enhanced HIF(s) transcriptional activation, sustaining angiogenesis and progression[74] |
| TASK1 | Subset of nonsmall-cell lung | The channel current is inhibited in hypoxia[144] |
| TASK3 | Lung (H146 cells) | Maintains resting membrane potential[147] |
| VGSCs | Various | Increment of cell invasiveness[148] |
| $\alpha$-ENaC | Liver (HepG2 cells) | Hypertonic stress (340 $\rightarrow$ 450 mosM) reversibly increased the $Na^+$ conductance and proliferation[149] |



in controlling metabolic pathways and especially hypoxia is possible thanks to their physical interactions with adhesion molecules (such as integrins) and transporters, forming proper central hubs controlling downstream signaling. In particular, $K_v11.1$, which couples with $\beta1$ integrin, regulates a novel signaling pathway that sustains angiogenesis and progression in colorectal cancer. This pathway is triggered by $\beta1$ integrin-mediated adhesion and leads to VEGF-A secretion. $K_v11.1$ acts as a modulator recruiting and activating PI3K and Akt. In turn, this increases HIF-dependent transcription of VEGF-A and other tumor progression genes.[74]

Another example involving a $K_v11.1$-centered complex is that of $K_v11.1$-CAIX in clear cell renal carcinoma, which is also involved in sustaining hypoxic pathway (Lastraioli et al.; submitted; under review). In this scenario, targeting such macromolecular hubs with specific tools such as antibodies will offer great opportunities for both diagnostics and therapy.

Antibodies have become common and essential research instruments over the last 50 years, providing highly specific and versatile tools for a wide array of experimental applications in many fields. Furthermore, monoclonal antibodies and, more recently, recombinant antibodies have gained clinical applications for diagnosis and therapy of different diseases, including cancer.[150,151] Specifically, a new class on antibodies capable of binding two different antigens at the same time, thus allowing the recognition of two independent targets that might be crucial in the diagnostic and therapeutic setting is that of bispecific antibodies, in the format of single-chain variable fragment diabodies (scDb). scDbs conjugate bispecificity with the characteristics of antibody fragments (low molecular weight, high tissue penetration, and good clearance times). Our group has already developed a bispecific scDb antibody directed against the $K_v11.1$-$\beta1$ complex, selectively expressed in cancers.[152] Such antibody, once tested through immunohistochemistry on both colorectal cancer and PDAC tissue specimens, confirmed its specificity for the complex. Such tools could be directed at ion channel complexes involving cancer metabolism and/or hypoxia- related proteins with the undeniable advantage of simultaneous dual targeting. In this regard, it is known that $K_v11.1$ plays a significant role in controlling several aspects of the tumor establishment and progression, including pH regulation. As already noted, there is a cross talk between $K_v11.1$ and $\beta1$, which complex together.[152] Such interaction, in CRC, could involve NHE1, which is involved in the control of intracellular pH. Moreover, from the molecular point of view, $K_v11.1$-$\beta1$ and NHE1 form a macromolecular complex that is highly expressed in CRC, HCT116 cells.[153]

It has recently been shown that in clear renal-cell carcinoma, $K_v11.1$ and CAIX are complexed together along with $\beta1$ integrin, modulating several aspects of tumor progression (Lastraioli et al.; submitted; under review). Overall, all these results confirm the important role of $\beta1$-integrins in pH-regulating mechanisms, demonstrating that $K_v11.1$ and NHE1 are involved in modulation of intracellular and extracellular pH, the latter having a role of ''mediator.'' In this scenario, it is worth validating recombinant bispecific or trispecific antibodies as possible tools to be applied for diagnostic and therapeutic purposes. Overall, the scDb–$K_v11.1$-$\beta1$ antibody could represent a potential new treatment tool, as well as an early molecular diagnostic marker, and thus could be an undeniable tool to target and decipher key actors of cancer metabolism [Duranti et al., unpublished data].

## Concluding Remarks and Future Perspectives

We have outlined here how metabolic changes in tumor cells reciprocally interact with tumor hypoxia, and how this involves major changes in ion channel expression and ion transport across the plasma membrane. Mounting evidence strongly indicates that these complex interconnections between ion channels, hypoxia, and the Warburg effect contribute significantly to cancer development, by promoting, for example, cell migration, invasion, and chemotherapy resistance. In this context, we suggest that the specific roles of ion channels may be exploited therapeutically. While the understanding of the roles and regulation of these ion channels in cancer has increased greatly in recent years, understanding the precise mechanism of ion channel-mediated hypoxic and metabolic reprogramming in tumors represents an intriguing

challenge. Future studies are warranted to address the precise mechanism of the role of ion channels in Warburg effect regulation. In turn, this could enable a move toward a possible clinical exploitation of the proposed evidence for the role of such proteins in cancer metabolism remodeling and tumor development.

### Authorship Confirmation Statement

J.I. designed and wrote the manuscript, performed bibliographical researches, and prepared the tables; G.P. designed and wrote the manuscript, performed bibliographical researches, and prepared the tables; C.D. contributed in writing the manuscript; E.L. wrote the manuscript and supervised it.

All coauthors have reviewed and approved of the manuscript before submission.

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