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Alban Girault, Ahmed Ahidouch, Halima Ouadid-Ahidouch. Roles for Ca²⁺ and K⁺ channels in cancer cells exposed to the hypoxic tumour microenvironment. *Biochimica et Biophysica Acta - Molecular Cell Research*, 2020, 1867, pp.118644 -. 10.1016/j.bbamcr.2020.118644 . hal-03489672

HAL Id: hal-03489672

<https://hal.science/hal-03489672>

Submitted on 21 Jul 2022

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Roles for Ca²⁺ and K⁺ channels in cancer cells exposed to the hypoxic tumour microenvironment

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Running title: Ca²⁺ and K⁺ channels in tumours under hypoxia

Abstract

For twenty years, ion channels have been studied in cancer progression. Several information have been collected about their involvement in cancer cellular processes like cell proliferation, motility and their participation in tumour progression using *in-vivo* models. Tumour microenvironment is currently the focus of many researches and the highlighting of the relationship between cancer cells and surrounding elements, is expanding. One of the major physico-chemical parameter involved in tumour progression is the hypoxia conditions observed in solid cancer. Due to their position on the cell membrane, ion channels are good candidates to transduce or to be modulated by environmental modifications. Until now, few reports have been interested in the modification of ion channel activities or expression in this context, compared to other pathological situations such as ischemia reperfusion. The aim of our review is to summarize the current knowledge about the calcium and potassium channels properties in the context of hypoxia in tumours. This review could pave the way to orientate new studies around this exciting field to obtain new potential therapeutic approaches.

Keywords: Ca²⁺ channels; K⁺ channels; Cancer; HIFs; Tumour hypoxia

Abbreviations:

AKT: protein kinase B; AP-1: Activator Protein 1; ARNT: Aryl Hydrocarbon Receptor Nuclear Translocator; ATP: Adenosine Triphosphate; BKCa: Big conductance Ca²⁺-activated K⁺ channel; Ca²⁺: Calcium; Ca²⁺ channels: calcium channels; [Ca²⁺]: intracellular calcium concentration; CBP: CREB-Binding Protein; CLIC: Chloride Intracellular Channel; COX-2: Cyclo-Oxygenase 2; DFO: desferrioxamine; DMOG: Dimethylxalylglycine; EGFR: Epidermal Growth Factor Receptor; EMT: Epithelial Mesenchymal Transition; ER: Estrogen Receptor; H₂S: Hydrogen Sulfide; hEAG: human Ether-à-go-go K⁺ channel; hERG: human Ether-à-go-go-Related Gene; HIF: Hypoxia Inducible Factors; HRE: Hypoxia Response Element; IKCa: Intermediate conductance Ca²⁺-activated K⁺ channel; K⁺ channels: potassium channels; K_{ATP}: ATP-sensitive K⁺ channel; Kv: Voltage-activated K⁺ channel; NFAT: Nuclear Factor of Activated T-cells; NFκB: Nuclear Factor Kappa B; PAS domain: Per-Arnt-Sim domain; PHD: Prolyl Hydroxylase; PIM kinase: Proviral integration site for Moloney murine leukemia virus-1 kinase; PTEN: Phosphatase and TENsin homolog; ROS: Reactive Oxygen Species; SKCa: Small conductance Ca²⁺-activated K⁺ channel; αSMA: alpha Smooth Muscle Actin; SOC: Store Operated Channel; SOCE: Store-Operated Calcium Entry; TASK: TWIK-Related acid-sensitive K⁺ channel; TGF-β: Transforming Growth Factor-Beta; TM: Tumour Microenvironment; TRP: Transient Receptor Potential; VEGF: Vascular Endothelial Growth Factor; VHL: Von Hippel-Lindau; VOCC: Voltage-Operated Ca²⁺ channel.

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Introduction

Tumour progression studies were for a long time restricted to the proper study of the cancer cells. However, conceptual progresses expanded the field of cancer analyses to a large set of elements named tumour microenvironment (TM) including extracellular matrix components, physical parameters like pH or oxygen level, stromal and immune cells hijacked from their function. Tumours are now largely considered like complex and heterogenic organs [1]. More especially, the high rate of cancer cell proliferation requires a high level of O₂ that cannot be supported by the blood flow supply, inducing a hypoxic environment. This particular condition triggers multiple cellular responses predominantly driven by the hypoxia-inducible factor 1 (HIF-1), which promotes cancer evolution [2]. Additionally to this stress status, the interactions between cancer cells and the other components of the TM increase the complexity and reduce the potential benefits of the clinical tools currently used. In this direction, Wu *et al.* recently reviewed the relation between the stroma in the tumor and the modulation of the therapeutic response [3]. For instance, it has been shown, in the context of the castration-resistant prostate cancer, that laminin could promote the resistance to PI3K inhibitors by stimulating the $\alpha6\beta1$ -integrin [4]. Moreover, the authors demonstrated in the same model that hypoxia could reinforce this resistance process by activating PIM kinase (Proviral integration site for Moloney murine leukemia virus-1 kinase). Despite major progress in the comprehension of this relationship, deep understanding of the communication between TM and tumour cells could be necessary to develop new therapeutic strategies.

A major class of actors well characterized that respond to the oxygen variations in cardiovascular and pulmonary systems is the ion channels' family [5]. Ion channels are fundamental proteins in living cells that control excitability, volume variation, and ion homeostasis. Their crucial roles make them major target in numerous pathologies. Moreover, their expression and their functions have been reported to be altered in numerous cancers and they have been described as the new hallmarks of oncochannelopathies [6]. In this context, it was demonstrated that they are regulators of cell motility and proliferation, and are also implicated in treatment resistance [6, 7]. Ion channels are located in several cell membranes (plasma membrane, organelle membrane and nuclear membrane), and are good candidates to sense and transduce information between the different compartments.

To date, few reports described the implication of the ion channels and more especially potassium and calcium channels in the response to hypoxia in tumour context. The aim of this

review is thus to highlight the relationship between these classes of proteins and the low oxygen context observed during cancer development.

Hypoxia in cancer

A common characteristic of tumours is low availability of oxygen called hypoxia, which is present under different forms of exposure: intermittent or chronic. Initially, uncontrolled cell proliferation exceeds the supply capacity of the blood flow. In addition, the distance between cells and blood vessels increases provoking a reduction of the O₂ diffusion and consequently a more hypoxic context. Moreover, transient obstruction of the dysfunctional blood network (due to cell aggregates) will be associated to a transient period of low O₂ supply. In response to this environmental modification, cancer cells will present different types of adaptation: induction of neo-vessel production, metabolism adaptation, survival promotion, metastasis and resistance to chemotherapy (**Figure 1A**) [8, 9].

A major driver of the answer to hypoxia is the family of heterodimeric hypoxia-inducible factors composed of one constitutive nuclear subunit HIF-1 β and one cytoplasmic oxygen-sensitive (HIF-1 α , HIF-2 α or HIF-3 α) [10]. More precisely, HIF α subunits are stabilized in O₂ decreased conditions by the inhibition of oxygen and iron-dependent enzymes named hypoxia-inducible factor-prolyl hydroxylase domain enzymes (PHD1-3). In the presence of O₂, hydroxylated HIF α subunits will be destroyed by the proteasome after ubiquitination *via* the von Hippel-Lindau tumor suppressor protein (pVHL) [11]. In decreased O₂ pressure condition, the stabilization occurs quickly (it only takes within a few minutes) and promotes the translocation of the HIF α subunits to the nucleus. This event allows the association with HIF-1 β , histone acetylases p300 and CBP, and consequently the fixation to their Hypoxia Responsive Element (HRE) to favour the expression of numerous genes involved in the processes described previously (**Figure 1B**) [12]. It is interesting to note that some cell responses to hypoxia are driven by other oxygen sensors like NF κ B, AP-1 or Early growth response gene-1 (Egr-1) [13-16].

Hypoxia context introduces numerous other modifications in the tumour progression as clonal selection, acid pH environment surrounding cells, or metabolism modifications.... Although hypoxia could provoke apoptosis [17] or necrosis of normal as well as cancerous cells, this situation usually increases aggressiveness of the tumour cells by hijacking proliferation, metabolism or angiogenesis pathways. Indeed, cells develop new phenotypes to protect themselves from the environmental stress: they acquire migration/invasion properties

[18, 19], stem cell capacities [20, 21] and resistance to currently used therapeutic tools like chemotherapy or radiotherapy [22-24]. In addition, cancer cells are rehabilitating to survive in their specific microenvironment. In order to adapt their metabolism, ATP production is switched from oxidative phosphorylation to glycolysis, the so-called Warburg effect [25]. This adaptation is largely driven by HIF-1 α , which induced, at less in part, the expression of transporters and enzymes necessary to this process. A direct consequence of this metabolism modification is a lactate overproduction inducing acidification of the closed environment of cancer cells [26]. The association of these mechanisms are thus involved in the poor prognosis associated to the hypoxia development [27, 28].

Ion channels and cancer

Ion concentration differences between both sides of the cellular membrane are crucial to the genesis and the regulation of numerous physiological functions. Indeed, flows of Na⁺, Ca²⁺, K⁺, Mg²⁺ or Cl⁻ are necessary to modify the electrical surrounding and the modulation of different cellular parameters. This flux is allowed by the ion channels, which are trans-membrane proteins designed especially to permit the passage of ions from one compartment to the other. Initially described in the excitable cells (neurons and muscle cells), ion channels are also crucial in the non-excitable cells to regulate cell volume, water flux, secretion, activation state or cell proliferation and death. Their ubiquitous expression makes them essential to the good function of the organism. Consequently, this class of protein is involved in numerous pathologies [29-32]. In addition, their deregulated expression and/or function are now classically described in cancer [6]. Ion channels have been described to control proliferation, survival or migration/invasion processes of cancer cells [33]. Nevertheless, little is known about the link between this class of protein and the hypoxic environment in cancer cells.

Involvement of Ca²⁺ and K⁺ channels in processes induced by hypoxia

Calcium channels

Many studies have highlighted the importance of Ca²⁺ in hypoxia induced cancer cell proliferation, survival, migration, angiogenesis, and invasion [34-36]. Indeed, in many cancer cell lines, chemical hypoxia procedures (Cobalt, Dimethylxalylglycine (DMOG) as well as 1

% O₂ exposition) lead to intracellular Ca²⁺ concentration ([Ca²⁺]_i) increase consecutively to Store-Operated Ca²⁺ entry (SOCE) enhance or basal entry. Otherwise, both Orai, Transient Receptor Potential (TRP), voltage-operated calcium channels (VOCC) are overexpressed in several cancer cell lines and regulate proliferation, survival, migration and invasion [37, 38]. However, despite the fact that hypoxia increases the [Ca²⁺]_i, the mechanisms linking hypoxia to TRP/Orai/ VOCC activation/expression in cancer are still emerging.

Hypoxia-induced intracellular Ca²⁺ elevation through upregulation of TRP / Orai and VOCC channels

Hypoxia is frequently associated to intracellular Ca²⁺ increase. Breast cancer cells exposed to hypoxia showed increased Ca²⁺ entry that is reduced by SKF-96365 and Orai1 or TRPC1 silencing. Interestingly, Orai1, TRPC1 are more expressed in hypoxia than in normoxia conditions [39-41]. In these cells, both TRPC1, which is not a SOC but a constitutively active channel, and Orai1, which is a SOC, contribute to Ca²⁺ entry and increase [Ca²⁺]_i. Silencing of TRPC1 and Orai1 reduce the constitutive Ca²⁺ entry and the SOCE respectively [40]. Additionally, hypoxia was also shown to upregulate Orai1 expression and support high SOCE and high [Ca²⁺]_i in colon cancer cells [42].

TRPC6 channel is considered a key player in hypoxia in different cancer types [43-46]. Two studies have reported the increase of [Ca²⁺]_i by hypoxia in glioma. However, the recorded Ca²⁺ intracellular increase is different depending on glioma cell lines. Using U-251 MG and U-87 MG glioma cell lines, Li *et al.* demonstrated an oscillatory profile, but they failed to record the same profile in U373 MG cell line [45]. In all cell lines, TRPC6 expression increased in hypoxia and induced a sustained basal Ca²⁺ concentration. TRPC6 knockdown (shRNA or negative dominant), or its pharmacological inhibition (SKF96365) suppressed both the hypoxia-induced [Ca²⁺]_i elevation, and the OAG-stimulated Ca²⁺ entry, but failed to affect the hypoxia-induced Ca²⁺ oscillation patterns. In regard to these data, it was reported that TRPC6 is responsible for the basal Ca²⁺ entry induced by hypoxia [43, 45]. Moreover, hypoxia increased also the TRPC6 expression and Ca²⁺ entry in human hepatocellular carcinoma cells [47], hepatic [44], and pancreatic stellate cells [46].

Recently, Monteith team reported the up-regulation of Orai3 expression in response to hypoxia in breast cancer cell lines (MDA-MB-468: ER⁻; basal A type, and MCF-7: ER⁺ luminal type), colon (HT29) and prostate cancer cell line (DU 145) without affecting the expression of Orai2 or Orai1 [39]. In contrast to Orai1, which constitute a SOC channels in

MDA-MB-468 cells, silencing of Orai3 failed to affect either SOCE or spontaneous Ca^{2+} oscillations observed in these cells during hypoxia treatment.

The majority of the studies show the hypoxia-induced overexpression of TRP- (TRPC1, and TRPC6), and Orai channels (Orai1 and Orai3). However, few studies have reported the signaling pathway involved. Two pathways seem to be involved: Notch and HIF-1 α .

Breast, colon and glioma cancer cells and activated hepatic stellate cells (stromal specialized from the hepatic tissue already described to participate to tumour processes [48]) when exposed to hypoxia, show increase in Orai1- [41, 42] and TRPC6 expression [43, 44], as well as Ca^{2+} entry that is associated to an increase in Notch (particularly Notch1) and its activation. In all cases, pharmacological inhibition or silencing of Notch1 decreased expression of both Orai1 and TRPC6 leading to reduction of SOCE and basal or OAG-activated Ca^{2+} entry suggesting the involvement of Notch signaling in the overexpression of these channels under hypoxia. In breast cancer, Monteith and his team have clearly showed the TRPC1 and Orai3 up-regulation through HIF-1 α [39, 40]. Indeed, silencing of HIF-1 α , but not HIF1- β or HIF-2 α , reduced both TRPC1 and Orai3 expressions. HIF-1 α was demonstrated to bind Orai3 promoter in MCF-7 cells [39], and binding sites of both HIF-1 α and HIF-1 β have also been identified in TRPC1 promoter region [40].

Hypoxia was also associated with an increase of voltage-operated calcium channels mostly T-type. In fact, both Cav3.1 and Cav3.2 isoforms are overexpressed in melanoma cancer [49] and positively correlated with glucose transporter (GLUT1), a hypoxia marker [50]. The overexpression of Cav3.2 was also shown in glioblastoma under hypoxia [51]. Hypoxia can also modulate the expression of membrane receptors, such as purinergic receptors. Indeed, in breast cancer, hypoxia changes the expression of both P2X and P2Y isoforms with a decrease of P2X4, P2X5, P2X7, P2Y1, P2Y11 and an increase of P2Y6 expression [52].

Crucial role of TRPC, Orai, and VOCC in cancer fates under hypoxia: signaling pathways

In basal-like triple negative MDA-MB-231 and BT549 breast cancer cells, hypoxia increased migration, invasion, and angiogenesis that involve the NFAT signaling pathway [41]. Indeed, by activating Notch1 signaling pathways, hypoxia increased expression of Orai1 and therefore increased SOCE that activates NFAT (NFAT4), which in turn, regulates invasiveness and angiogenesis [41]. The same authors reported hypoxia increased colon invasiveness and angiogenesis by regulating Notch1/Orai1/SOCE/NFATc3 [42]. In another

basal-like MDA-MB-468 triple negative breast cancer cells, hypoxia induced migration, EMT (epithelial-to-mesenchymal transition) and autophagy [40]. TRPC1 promotes EMT, through HIF-1 α -dependent pathway. TRPC1 silencing decreases the expression of the mesenchymal marker SNAIL1, and partially increased the level of the epithelial one, CLDN4. TRPC1 also increases the autophagy marker levels (LC3BII), *via* transactivation of EGFR and regulates hypoxia-induced STAT3 activation. In contrast to TRPC1, silencing of Orai3 failed to affect both hypoxia-induced EMT and migration in MDA-MB-468 cells suggesting it's no involvement in these two processes. The authors suggest that Orai3 may regulate the expression of genes involved in inflammatory and immune responses in breast cancer [39]. Hypoxia was also associated with a reduction of the sensitivity of MDA-MB-468 to extracellular ATP stimulation and an increase of P2Y6 isoform. Silencing of P2Y6 or its pharmacological inhibition attenuates hypoxia-induced vimentin expression protein level suggesting a role in the hypoxia-induced EMT in breast cancer [52].

In A549 lung cancer cell lines, hypoxia promotes COX-2 expression and favors migration and invasion [53]. Pharmacological activation of TRPA1 by Methyl syringate completely suppressed COX-2 expression and inhibited migration and invasion under hypoxia conditions. Furthermore, pharmacological activation of TRPM8 (Menthol) favors cell growth and invasion of prostate cell line overexpressing TRPM8 [54] and this effect is inhibited when silencing of TRPM8.

TRPC6 is the most studied channel in term of cancerous effects induced by hypoxia. It is considered as a key cellular response to hypoxia. Thus, in pancreatic stellate cells (an important class of stromal cell involved in the pancreatic cancer development [55]), TRPC6, by increasing $[Ca^{2+}]_i$, is involved in migration induced by hypoxia likely by increasing/maintaining secretion of cytokines by which stellate cells are stimulated in an autocrine fashion [46]. It was also shown in human hepatic stellate cells that the increased expression of TRPC6, under hypoxia, leads to Ca^{2+} entry that activates NFATc1 resulting in the increase of expression of α SMA and collagen, two mediators of fibrotic activation [44]. Moreover, TRPC6 by activating SMAD2/3 dependent-TGF- β signaling pathway promotes the up-regulation of expression of α SMA and collagen. Silencing of TRPC6 reduced the expression of both α SMA and collagen suggesting that, under hypoxia conditions, TRPC6 is involved in the switch from the quiescent to activation state of hepatic stellate cells. TRPC6 is overexpressed in hypoxic condition in glioma cell line (U-373MG) and primary human glioma and regulates cell proliferation, angiogenesis, and invasion in NFAT-dependent

manner [43]. In other glioma cell lines (U-251MG and U-87MG), under hypoxic conditions, the activation of TRPC6 through IGF1R-PLC γ -IP3R pathway lead to a calcium entry that controls HIF-1 α accumulation. However, this phenomenon seemed unrelated to its transcription or translocation. TRPC6 also controls GLUT1 expression and glucose uptake. This control is operated through HIF-1 α under hypoxic conditions with no effect on lactate level [45].

The role of TRP channels in hypoxia-induced chemoresistance has been also reported. It's the case of TRPC6 that is involved in resistance to chemotherapy treatments notably that induced by doxorubicin under hypoxic conditions. Indeed, silencing of TRPC6 significantly reduced this drug resistance [47].

Some work has focused on the role of VOCC in hypoxic conditions. In glioblastoma cells, Cav3.2 knockdown or its pharmacological inhibition (mibefradil) increases cell death and decreases the expression of HIF-1 α and HIF-2 α suggesting the regulation of glioblastoma cell survival by Cav3.2 through HIF-1 α and HIF-2 α [51]. Under moderate hypoxic conditions (2% O $_2$), Das *et al.* showed an overexpression of Cav3.1 and Cav3.2 isoforms only in high rate proliferation melanoma cell lines (JG and M2). Moreover, silencing of both isoforms reduced cell proliferation by accumulating cells in G1 phase and reducing their percentage in S phase [49].

A summary of these works is presented in **Table 1** and in **Figure 2**.

Potassium channels

The involvement of potassium channels in response to PO $_2$ variation in the carotid body has early been described [56]. The reduction of oxygenation level induces inhibition of K $^+$ channels provoking depolarisation in order to elicit the release of transmitters. The physiological answer is hyperventilation to restore the O $_2$ pressure. In the neuroepithelial bodies located in the lung, similar K $^+$ channels' function have been described [57]. Directly derived from these first observations, the roles of K $^+$ channels in pathological processes involving hypoxia periods such as cardiac or neuronal ischemia have been largely studied. In these conditions, it has been shown, for example, that BKCa (Big conductance Ca $^{2+}$ -activated K $^+$ channel) or SKCa (Small conductance Ca $^{2+}$ -activated K $^+$ channel) channels could participate to the preservation of tissue by regulating the Ca $^{2+}$ influx at the subcellular level or by promoting vasoconstriction in order to optimize the gas exchanges [58]. Similarly, K $_{ATP}$ channel, which is modulated by the ATP/ADP ratio, is activated during cardiac ischemia and

has been shown to allow cardiomyocytes protection through different signaling pathways [59]. This field has recently been extended to cancer.

Two-pore-domain potassium channels (K2P channels)

Since almost fifteen years, different studies report the relationship between hypoxia (or hypoxia-induced processes) and K^+ channels in cancer cells. First, Mu and collaborators studied a specific locus amplified in numerous breast cancer cases [60] and they found that the gene located in this place coded for the TASK-3 channel (*KCNK9*). They then demonstrated that this channel is able to participate to the promotion of the tumour progression and the viability of cell culture in serum-reduced condition or in hypoxia. Thereafter, this channel has been shown to participate to survival and proliferation of breast cancer cells without connection to hypoxia [61, 62]. However, the studies demonstrated that TASK-3 could also be located at the mitochondrial level, suggesting an important role in the regulation of apoptosis. The TASK-1 channel, a structurally close channel that is regulated by pH variation, has also been studied in the context of hypoxia in lung cancer cells [63]. It has been demonstrated that cells exposed to hypoxia presented a TASK-1 current reduced without effect on the RNA or protein quantities and the author suggested that this channel could thus be an oxygen sensor to protect cells in a low perfused area and could promote proliferation in presence of proper conditions. The results from these studies could indicate that these K2P channels are able to participate to the protection process in hypoxia to allow cancer cells to survive in inadequate environment.

ATP-sensitive potassium channels

K_{ATP} channel, which has been well described in O_2 sensing in the vascular and cardiac systems, has also been studied in cancer. By using a human hepatoma cell model (HepG2 cells), Zhang *et al.* demonstrated that K_{ATP} channels are involved in hypoxia-induced radioresistance [64]. More precisely, they showed that irradiation induced the production of hydrogen sulphide (H_2S) synthases and consequently increased the resistance to radiotherapy. Additionally, concomitant treatment of a hydrogen sulphide donor with glibenclamide, a K_{ATP} inhibitor, restored the radiosensitivity demonstrating that the H_2S could activate K_{ATP} and prevent irradiation-induced death of tumour cells.

Calcium-activated potassium channels (KCa channels)

The family of calcium-activated potassium channels has also been demonstrated to participate to the response of cancer cell to hypoxia. This class of K^+ channels is subdivided

in three ramifications depending of their biophysical and pharmacological properties: the small conductance (SK1, SK2 and SK3), the intermediate conductance (IKCa also named SK4, KCa3.1, or IK1) and the big conductance (BKCa, Maxi-K, slo1 or KCa1.1). BKCa channel has been largely studied in the pathological context of glioma and has been described at the plasma and at the inner mitochondrial membranes. Gu and his collaborators demonstrated that mitochondrial BKCa activity is increased in hypoxia although the plasma channel is not affected [65]. Surprisingly, the positive modulation of the inner mitochondrial membrane doesn't affect glioma cell's viability while stimulation of the plasma membrane BKCa induced propidium iodide uptake and decreased ATP metabolism in normoxia *versus* two more times severely in hypoxia. By completing their point of view through a commentary about their own article, the authors suggested that BKCa channel position at the plasma membrane is an adaptation to the glioma cell survival in stress conditions like hypoxia and escapes the cells from the volume-dependent apoptosis [66]. In melanoma models (cell line and metastatic isolated primary cells), Tajima *et al.* demonstrated that hypoxia or hypoxia mimetics (CoCl₂ and desferrioxamine (DFO)) could modulate IKCa and SK currents [67]. To a greater extent, they show that hypoxia increases charybdotoxin- (IKCa inhibitor) and apamin (SK inhibitor)-sensitive currents in the IGR-1 cells. Moreover, the use of these pharmacological compounds reduced the hypoxia-induced proliferation of the cells.

Voltage-gated potassium channels

Voltage-gated K⁺ channels constitute the largest branch of K⁺ channels. This class has been largely studied in excitable structures like neurons and cardiac muscle cells but they are also involved in different tumour processes. In the context of cancerous hypoxia, several studies have reported an important function of this subfamily.

hERG channel has been largely described in numerous types of cancer and has been involved in cell proliferation, migration/invasiveness, and the regulation of neoangiogenesis [68]. It has early been reported that hypoxia modifies the activity of hERG channel in SH-SY5Y neuroblastoma cells [69]. In this work, authors described that the electrophysiological properties of the aforementioned channel are modified: slow-down of the inactivation phase, hyperpolarisation of the voltage-dependence activation and a shift of the window-current closed to the resting potential of cancer cells. By using the same cellular model, the Nanduri's group found conflictual results [70]. They demonstrated that mature hERG channel is decreased when cells are exposed to chronic hypoxia and then consequently the current is reduced leading to a proliferation diminution. Finally, in digestive cancer, Arcangeli's group

highlighted a relationship between hERG, hypoxia and neo-angiogenesis (a relationship between hERG channel and HIF in normoxia is also reported through these studies and will be discussed in the latter section) [71, 72]. Initially, they showed that hERG channel, by interacting with β 1-integrin, regulates VEGF secretion in colorectal cancer cells exposed to hypoxia [72]. They confirmed their results with preclinical models in which they compared the vascularity index between animals receiving cells expressing or not hERG. In a second time, they demonstrated that hERG channel could become a good prognostic marker of gastric cancer evolution [71]. In the latter case, authors evaluate the association of hERG expression with clinical and prognostic markers of gastric cancer. Moreover, they correlated the pharmacological inhibition of hERG with the reduction of vascularization in *in vivo* models.

hEag1 is a closely related channel to hERG but presents different pharmacological and electrophysiological profiles. hEag1 has been among the first K^+ channels described in the development of tumour process [73]. Two studies reported a relationship with hypoxia. First, Downie and collaborators presented the interesting result that cells expressing functional hEag1 provoked a faster growth in nude mice and these tumours showed high rate of vascularization [74]. In addition, they demonstrated that hEag1 expressing cells have more HIF-1 α expression in presence of hypoxia suggesting the involvement of the channel in the regulation of the angiogenesis. Second, an observational study demonstrated that hEag1 presented a high correlation with the expression of HIF-1 α in an Asian breast cancer cohort [75].

Other representatives of this family have been implied in tumour progression under hypoxia. For instance, Kv3.1 and Kv3.4's expressions increased in hypoxia mimicking conditions and their inhibition reduced the A549 (lung cancer), MDA-MB-231 (breast cancer) and HT29 (colorectal cancer) migration capacities [76]. In addition, Blood Depressing Substance (Kv3.1 and Kv3.4 inhibitor) reduced invasion properties of A549 and MDA-MB-231 cells without affecting the proliferation of the different cell lines tested. Ryland and collaborators also demonstrated that Kv1.5 could be involved in apoptosis regulation of Erwing sarcoma or neuroblastoma in hypoxia condition [77]. The role of this channel in response to hypoxia has already been well reviewed by Archer *et al.* at the interface of pulmonary arterial hypertension (PAH) and cancer [78]. Briefly, a relationship between mitochondrial function and altered Kv1.5 expression or function should be involved in PAH and cancer to promote vasoconstriction, apoptosis deregulation or abnormal proliferation. In

another way, medulloblastoma cells express functional Kv2.1 channel that is regulated by heme oxygenase and promote resistance to apoptosis [79].

Signaling pathways involved in hypoxia-induced K⁺ channel modifications

Although the comprehension of the K⁺ channels implication in response to hypoxia in cancer cells is quickly improving, little information is available about the intracellular processes engaged. Regarding K⁺ channels, two major ways emerged in this context: K⁺ channels are directly affected by O₂ pressure variation or K⁺ channel expression / function is modified in downstream of another O₂ sensors such as HIF family (**Figure 3**).

It was initially described that hypoxia reduced K⁺ channel activity in alveolar model of neuroepithelial body [80] or PC12 pheochromocytoma cells [81]. Since these first evidences of the hypoxia inhibitory effect on K⁺ channel activity showed in the characterization study models of vascular and respiratory system, several groups reported similar activity in numerous models. For example, BKCa channel expressed by glioma cells, presented different sensitivity to hypoxia depending its cellular localization (plasmalemma or mitochondrial membrane) but authors did not present specific mechanisms [65]. It has also been shown that hypoxia could reduce TASK1 activity in lung cancer cells, inducing modification of the expression profile of different nutrient transporters, promoting the resistance of cancer cells to environment stress [63]. Fontana and her colleagues showed that hERG channel activity could be modulated by hypoxia without HIF implication and suggested that hypoxia acts directly on the channel through its PAS domain or by the mean of another O₂ sensor [69].

Several years later, it has been surprisingly demonstrated that HIF-1 α in response to hypoxia is implicated in the promotion of the KCa channels activity in melanoma cells demonstrating that cancer cells could adapt their response to surpass stress conditions [67]. In a medulloblastoma cell model, it has also been shown that other actors could modulate K⁺ channel activity [79]. In this work, authors demonstrated that cell exposition to hypoxia induced the expression of the heme-oxygenase 1, which produces CO. This gas acts as mediator and inhibits Kv2.1 channel and promotes apoptosis resistance.

We recently reviewed the role of ion channels in the chemoresistance in cancer [7]. However, it has also been reported that ion channels could participate to cancer cell radioresistance [82]. In addition, hypoxia is also known to reduce the sensitivity of cancer cells to radiotherapy by decreasing the O₂ availability required to the therapeutic action of irradiation [83]. In this context, Zhang *et al.* demonstrated that ionizing radiation induced the expression of enzyme producing hydrogen sulfide [64]. They then showed that hydrogen

sulfide (from endogen origin or from donor compound) increased K_{ATP} activity and promoted apoptosis resistance of hepatoma cells.

Another way to modify the role of ion channels in cells is to modify their expression. This process has also been described in cancer context. By using different model cells (A549 from lung cancer, MDA-MB-231 breast cancer cells and HT29 issued from colorectal cancer), Song demonstrated the involvement of the Kv3.1 and Kv3.4 channels in migration/invasion processes as previously described [76]. More precisely, their work showed that the regulation of the Kv channel-dependent cellular processes is based on the ERK pathway, which is already known to participate at the regulation of HIF-1 α protein synthesis. In neuroblastoma cells, hERG channel has been engaged in the cell cycle arrest induced by hypoxia [70]. This study showed that hypoxia reduced the production of hERG mature form. This effect should be due to the retention of the channel in endoplasmic reticulum, where the increase of hypoxia-induced ROS modified the capacity of hERG channel to interact with HSP90 and finally disrupted its trafficking to the membrane. Regarding the Kv1.5 channel an epigenetic regulation of its expression has been reported to be important to protect the Erwing sarcoma and neuroblastoma cells from apoptosis [77]. Through this work the authors showed that both the tumor cell types overexpressed the polycomb group protein BMI-1 that repressed the Kv1.5 expression promoting the survival of the cells.

More and more evidence are demonstrating that ion channels do not work alone but are involved in large protein complexes. One of the best-characterized systems is the association of hERG channel with the integrin in different cancer models (for review [84]). A recent study from Arcangeli's team demonstrated this by using colorectal cancer (cellular and preclinical) models. They showed that hERG, in association with the β 1-integrin, regulated the signaling pathway involving AKT, NF κ B and HIF-1 α to promote the VEGF-A secretion [72]. Although this mechanism exists in normoxia, it is important to note that the signal is amplified in hypoxia and in p53 KO cell model.

Another form of complex is the ion channels' association. A logical link is the relation between KCa channels and Ca²⁺ channels, which is previously reviewed by Guéguinou *et al.* [85]. Mignen and collaborators recently updated the importance of basal Ca²⁺ entry to regulate tumour progression including the processes involving K⁺ channels [86]. Briefly, KCa activation drives a hyperpolarization that consequently increases the driving force of Ca²⁺, which passes through Ca²⁺ channels to enter the cell and to enhance intracellular Ca²⁺ concentration. This latter augmentation promotes the KCa activation and participates to an

auto-sustained activation loop. This type of relation has been well described in different models such as breast, colon or prostate cancer cells [87-89]. Despite the current lack of evidences, this interaction could be another putative way of the ion channels' participation in the response to hypoxia. Additionally to the direct link between KCa channels and Ca²⁺ channels, it has also been reported the interplay of other K⁺ channels as hEag1 with Orai1 channel in breast cancer cell model [90]. In the context of hypoxia, a deepening of studies based on these relationships could bring a great added value to the understanding of the role of ion channels in the interconnection between cancer cell and TM.

A short summary of the different mechanisms involving K⁺ channels in the cancer cell responses is reported in the **Table 2**.

Other ion channels and transporters in hypoxia tumour context

The channels described above are not the ones involved in the response to hypoxia of cancer cells. The focus of our review is the role of Ca²⁺ and K⁺ channels in the relation between hypoxia tumour condition and cancer cell responses. Nevertheless, other studies report the involvement of chloride and sodium channels and different classes of transporters such as aquaporin, NMDA or acetylcholine receptors.

Indeed, chloride channels are also involved in this cellular process. For example, Sforza and collaborators demonstrated that the swelling-activated chloride current (ICl_{swell}) could be activated by prolonged hypoxia [91]. This activation could prevent the necrosis cascade induced by the swelling and could also suggest that hypoxia-induced stimulation of ICl_{swell} participates to cell survival. The chloride intracellular channel 1 (CLIC-1) could also participate to the hypoxia-induced response of colorectal cancer cells [92]. By using CLIC-1 inhibitors, authors demonstrated that channel inhibition reduced the hypoxia-induced ROS production, cell invasion and migration, and ERK pathway activation. Additionally, they demonstrated that the CLIC-1/ROS/ERK axis regulated the MMP-2/MMP-9 production, crucial enzymes for metastasis formation.

Different groups also reviewed the implication of ASIC channels or Na⁺ current in the context of tumour hypoxia [93, 94]. The relation between these classes of ion channels, the microenvironment and the cancer cell response is getting better understood. These reports in association with this current work thus suggest the importance of this research field.

In addition to ion channels, numerous receptors and transporters have been described to regulate hypoxia-mediated processes. Aquaporins, a large class of water transporters, have

been involved in glioma, prostate, breast or NSCLC cancers [95-98]. It is interesting to note that the mechanisms implicated in this class of proteins are close to the ones previously described for Ca^{2+} and K^+ channels. For example, Aqp3 has been described in the regulation of the NSCLC cell proliferation by regulating HIF-1 α /VEGF axis and the Raf/MEK/ERK signaling pathway [96]. The expression of this family could also be regulated in hypoxia context. For instance, Tie *et al.* demonstrated that hypoxia could increase $[\text{Ca}^{2+}]_i$, which in turn activated the protein kinase C, regulated p38 activity, and subsequently promoted the transcription of the Aqp1 in prostate cancer cells [97].

Several studies also reported the interactions of NMDA, GABA or acetylcholine receptors with tumour progression in hypoxia. The putative therapeutic use of these pathways has already been reviewed (e.g. β adrenergic system [99]).

Involvement of Ca^{2+} and K^+ channels in the regulation of HIF in normoxia

Hypoxia appearance is classically described as a progressive mechanism. For this reason, cancer cells must prepare to respond to this new condition. Adaptation processes are thus required and some reports present ion channel as actor of this cascade of events. The works present here will be summarized in **Table 3**.

Link between TRP and HIF-1 α in normoxia: adaptation process to hypoxia

Growing studies reported an upregulation of Ca^{2+} channels expression in a wide variety of cancers using both *in-vivo* models and human tissues [6]. These works have initiated others that proposed several Ca^{2+} channels as markers of different cancers. Several studies have also shown the overexpression of HIF-1 in many human cancer tissue types [100]. This factor has also been proposed as a potential prognostic marker for tumor [101].

We now know that some Ca^{2+} channels regulate the expression of HIF-1 α also under normoxic conditions. This is the case of TRPC1, which regulate HIF-1 α translation in thyroid cancer likely *via* PKC and Erk1/2 [102], and in breast cancer by modulating Akt activation [40]. Knockdown of TRPC1 reduces HIF-1 α expression, inhibits EMT in breast cancer and invasion/migration in thyroid cancer. In prostate cancer, TRPM8 overexpression subdues RACK1-mediated HIF-1 α ubiquitination and, consequently, increases HIF-1 α protein level [54]. Likewise, under normoxic conditions, the chemotherapy-induced TRPC5 up-regulation leads to nuclear HIF-1 α accumulation in breast cancer cells [103]. Immunofluorescence

studies on 42 tissue samples showed a positive correlation between TRPC1 and HIF-1 α in patients treated with chemotherapy. In colon cancer cells under normoxic conditions, TRPC5 overexpression leads to an EMT through HIF-1 α signaling pathway, which further upregulates both cell-proliferation and migration [104]. Chen *et al.* investigated the differential expression of TRPM2-L and TRPM2-S isoforms of TRPM2 in neuroblastoma and found a greater expression of the long isoform of TRPM2 (TRPM2-L) in contrast to the short isoform (TRPM2-S) that showed a little expression [105]. Functionally, TRPM2-S expressing cells found to show a down-regulation of HIF-1 α and HIF-2 α unlike those expressing TRPM2-L. Moreover, TRPM2-S-reduced cell proliferation and sensitive cells to the effect of doxorubicin likely through HIF-1/2 α .

It is now undoubtful that the overexpression of some Ca²⁺ channels regulates HIF isoforms under normoxic conditions. This may constitute a cellular adaptation process to hypoxia. The overexpression of these channels in cancer may thus allow cells the ability to adapt to hypoxia by initiating a special transcriptional program making them resistant to hypoxia.

Hypoxia mediators and potassium channels in normoxia

Even though it is now clear that K⁺ channels could participate in the response to hypoxia in cancer cell to promote the resistance to stress condition or to improve the tumour progression, there are evidence that this family of membrane proteins could also participate in the preparation of the cell to undergo these pathological states. We just now described the role of the hERG/ β 1-integrin in the colorectal cancer where this complex could modulate the expression of HIF-1 α in normoxia to promote the neo-angiogenesis [72]. The same group demonstrates in gastric cancer that hERG channel could also promote the VEGF-A secretion by modulating HIF signaling *via* an AKT-dependent mechanism in normoxia [71]. More precisely, they showed that HIF activity is inhibited by the use of hERG pharmacological inhibitors and they deciphered that the modulation is impacting the gene regulation by HIF-2 α and HIF-2 α /HIF-1 α but not only by HIF-1 α . Data issued from these works suggest that ion channels could participate to the adaptation of cancer cell to promote the aggressive profile.

In addition, it has been reported that the Aryl hydrocarbon receptor nuclear translocator (ARNT also named HIF-1 β) could modulate the Kir6.2 expression in insulinoma cell model [106]. In this study, the authors demonstrated that the control of the channel

expression is independent of the HIF-1 α or HIF-2 α proteins but they did not identify the partner(s) of ARNT involved in this regulatory mechanism.

Conclusions and perspectives

Hypoxia is always a barrier to obtain the best result of cancer therapies and its participation to the tumour progression is not yet fully understood. Numerous signaling pathways drive cancer cell modifications (e.g. apoptosis resistance, increase of cell proliferation/migration/invasion, therapeutic resistance...). Although the knowledge about the different pathways is permanently increasing, new alternatives will be necessary to improve the patient care and to preclude cancer evolution. Here, we reviewed the different investigations conducted about K⁺ and Ca²⁺ channels involved in the context of hypoxia, which highlight the importance of these proteins. It is also very important to note that ion channels and transporters are also implicated in parameters closely related to hypoxia as acidic pH environment or metabolism switch. Two recent reviews reported the importance of these proteins in these processes reinforcing their importance as potential therapeutic targets [107-109].

Although the number of studies about the role of ion channels in hypoxia is permanently increasing, it should be important to intensify the work about this interesting field. Additionally, it will be necessary to more describe the complete intracellular mechanisms in charge of the increase aggressiveness involving K⁺ and Ca²⁺ channels. Moreover, it is necessary to take into account that channels could act as complexes. Association of Ca²⁺ and K⁺ channels has already been described in migration or in survival mechanisms in cancer cells [87, 110]. Similar association could be detected in tumour cells exposed to hypoxia. Otherwise, we did not discuss very much the mitochondrial ion channels. Due to the role of this organelle in the regulation of cell metabolism and in control of apoptosis, which are both modified in hypoxia, it seems very important to enlarge the investigations towards this field of study.

In order to make to progress in this way, it should be important to improve the models used to evaluate the hypoxia effects by employing more complex tools like 3D culture support (including matrix components and cells from the microenvironment) [111] or organotypic models [112]. It is also crucial to integrate the role of ion channels in tumour progression by studying their role in concomitant processes like EMT, angiogenesis regulation, cancer stem cells properties acquisition or chemo-/radio-resistances.

To conclude, this emerging field will highlight new target opportunities to fight against cancer progression. A lot of work will be necessary to routinely use them in clinic but it would be important to evaluate the transfer of some ion channel modulators already developed (for cardiac, hypertension, neurologic pathologies) to improve current cancer treatments.

Acknowledgments

This work was supported by the UPJV “Université de Picardie Jules Verne”, the “Région Hauts-de-France” and the CNO (“Cancéropôle Nord-Ouest”).

Author statement

Conceptualisation: AG, AA, HOA; Writing (Original Draft, Review & Editing): AG, AA, HOA.

Conflicts of interest

The authors declare that they have no conflict of interest.

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Cancer model	Channel type	Effect	Signaling pathways	Hypoxia induction	Ref
Breast cancer MDA-MB-231, BT549	Orai1	Migration Invasion Angiogenesis	Notch activation, up-regulation of SOCE <i>via</i> up-regulation of Orai1, activation of NFAT4	1% O ₂	[41]
Colon cancer HCT-116, SW480 and HMEC-1	Orai1	Invasion, migration, angiogenesis	Notch activation, up-regulation of SOCE <i>via</i> up-regulation of Orai1, activation of NFATC3	CoCl ₂	[42]
Breast cancer MDA-MB-468, HCC1569, MDA-MB-231	TRPC1	EMT Autophagy	Increase SNAIL expression through HIF-1 α and decrease of CLDN4 (epithelial marker) Transactivation of EGFR	1% O ₂	[40]
Breast cancer MDA-MB-468, MDA-MB-231	Purinergic receptors P2Y6	EMT Migration	Increased P2Y6 expression partially <i>via</i> HIF-1 α increase vimentin expression	1% O ₂	[52]
Lung cancer A549	TRPA1	Invasion Migration VEGF secretion	Down-regulation of Cox2 expression and activity	1% O ₂	[53]
Prostate cancer LNCaP VCaP NCK-H660	TRPM8	Growth Invasion	Activation of TRPM8, inhibition of p-RACK-1, stabilization of HIF-1 α expression	1% O ₂	[54]
Pancreatic stellate cells	TRPC6	Migration	Increased TRPC6 expression, Increased Ca ²⁺ entry, Increased cytokine secretion	1% O ₂ 0.5 mM DMOG	[46]
Hepatic stellate cells Ix-2 cell line	TRPC6	Synthesis of extracellular matrix proteins (ECM)	- Up-regulation of TRPC6, through HIF-1 α , increased sustained [Ca ²⁺] _i , that regulate NFATC1 activation, which promotes ECM proteins expression (α SMA and collagen). - Facilitate the effect of TGF- β in regulating ECM synthesis through SMAD2/SMAD3 pathway.	100 μ M CoCl ₂	[44]
Glioblastoma U373MG, HMEC-1	TRPC6	Growth, invasion Angiogenesis	Up-regulation of TRPC6 <i>via</i> Notch1 pathway, sustained basal Ca ²⁺ entry, activation of NFAT	100 μ M CoCl ₂	[43]
Glioma cells U87 U-251	TRPC6	Metabolite levels and Glucose uptake	TRPC6 activation, through IGF-1/PLC γ pathway, Ca ²⁺ entry that accumulates HIF-1 α (decrease its hydroxylation) Increase GLUT1 expression	DMOG 1% O ₂	[45]
Hepatocellular carcinoma Huh7, HepG2	TRPC6	Drug resistance	Increase TRPC6 expression, sustained Ca ²⁺ entry Increase HIF-1 α expression likely <i>via</i> STAT3 activation	1% O ₂	[47]
Glioblastoma U87, A172, U373, T98C, SNB19	VOCC (Cav3.2)	Resistance to chemotherapy	HIF-1 α and HIF-2 α expression	1% O ₂	[51]
Melanoma JG M16 M28	VOCC (Cav3.1 and Cav3.2)	Cell proliferation (Cell cycle progression: S phase)	Up-regulation of Cav3.1 and Cav3.2 in fast growing cell lines: JG and M28 cells. Silencing both isoforms reduced cell proliferation and accumulated cells in G1/S phase.	2% O ₂	[49]

Table 1: Summary of Ca²⁺ channels involved in hypoxia-induced processes

Cancer model	Channel type	Effect	Signaling pathways	Hypoxia induction	Ref
NSCLC A549 A427 SK-MES-1	TASK-1	Apoptosis Proliferation Metabolism adaptation	Membrane potential regulation Increased expression of putative downstream effectors (SLC38A1, SLC5A3, SLC5A6)	1% O ₂	[63]
Melanoma IGR39 Isolated melanoma cells	KCa channels	Proliferation	Up-regulation of KCa currents in hypoxia	3% O ₂ CoCl ₂ DFO	[67]
Medulloblastoma DAOY	Kv2.1	Apoptosis resistance	Up-regulation of Heme Oxygenase 1 induces CO production, which inhibits Kv2.1	0.5% O ₂	[79]
Hepatoma cells HepG2	K _{ATP}	Hypoxia-induced radioresistance	Up-regulation of H ₂ S producing enzyme H ₂ S-increased K _{ATP} current	0.1% O ₂	[64]
A549 MDA-MB-231 HT29	Kv3.1 Kv3.4	Cell migration and invasion	Kv channels regulates Erk signaling pathway and modulation affects acidic compartments	Local hypoxia induced by cell density	[76]
Neuroblastoma SH-SY5Y	hERG	Cell cycle progression	Hypoxia reduces mature hERG by increasing ROS, which interfere in the association of hErg with HSP90 and decrease membrane traffic	1.5% O ₂	[70]
Erwing sarcoma cells (LA1-55N) Neuroblastoma cells (SH-SY5Y)	Kv1.5	Survival	Epigenetic regulation of Kv1.5 expression by the BMI-1 (from Polycomb group proteins)	1% O ₂	[77]
Colorectal cancer HCT116 HT8	hERG	Angiogenesis Tumour progression	Complex hERG - integrin β 1 regulates p85-Akt-NF κ B-HIF-1 α axis and promotes VEGF-A secretion Association with p53 activity	0.1% O ₂	[72]

Table 2: Summary of K⁺ channels involved in hypoxia-induced processes

Cancer model	Channel type	Impact Channel on HIF expression	Ref
Breast cancer	TRPC1	Regulates HIF-1 α translation <i>via</i> pAKT	[40]
Thyroid cancer	TRPC1	Regulation of HIF-1 α translation likely <i>via</i> PKC and Erk1/2 pathways	[102]
Prostate cancer	TRPM8	Induces HIF-1 α accumulation <i>via</i> dephosphorylation of RACK1, stabilization of HIF-1 α	[54]
Breast cancer	TRPC5	Favors HIF-1 α accumulation in the nucleus	[103]
Colon cancer	TRPC5	Increases expression of HIF-1 α	[104]
Neuroblastoma	TRPM2-L	Up-regulation of HIF-1 α and HIF-2 α	[105]
Colorectal cancer	hERG1	Up-regulation of HIF(s) transcriptional activation <i>via</i> PI3K/Akt signaling	[72]
Gastric cancer	hERG1	Main up-regulation of HIF-2 α transcriptional activation <i>via</i> an Akt-dependent pathway	[71]

Table 3: Impact of Ca²⁺ and K⁺ channels on HIF expression in normoxia

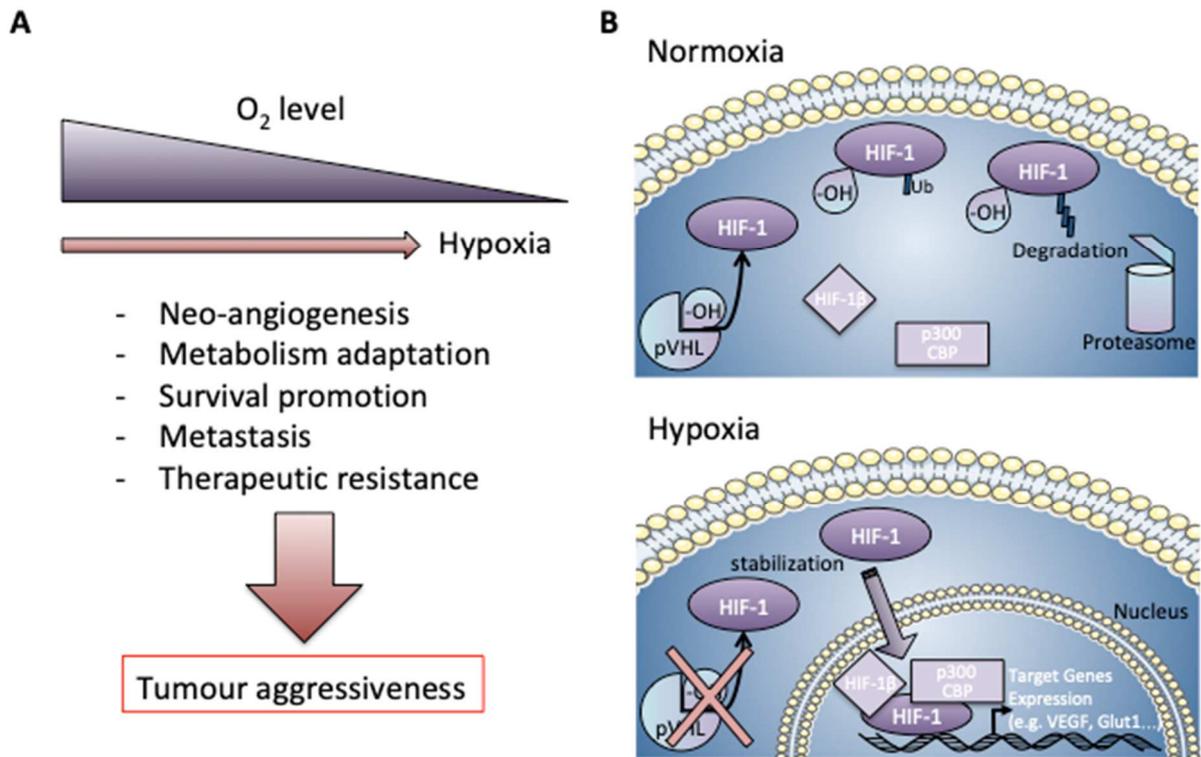


Figure 1. Hypoxia and HIF-1 subunits activity

A. Summary of the hypoxia-induced processes detected in cancer cells. **B.** Simplified scheme of the HIF-1 course in normoxia (top panel) and hypoxia (bottom panel). Briefly, in normoxia conditions, pVHL hydroxylates proline of HIF-1. After that, HIF will be ubiquitylated and degraded by proteasome. Hypoxia induces the stabilization of HIF-1 by inhibiting the activity of VHL. Consequently, HIF-1 is translocated to nucleus, where it will be associated with HIF-1 β , p300 and CBP, in order to induce the transcription of numerous effectors like VEGF, Glut1.... (Ub: ubiquitine).

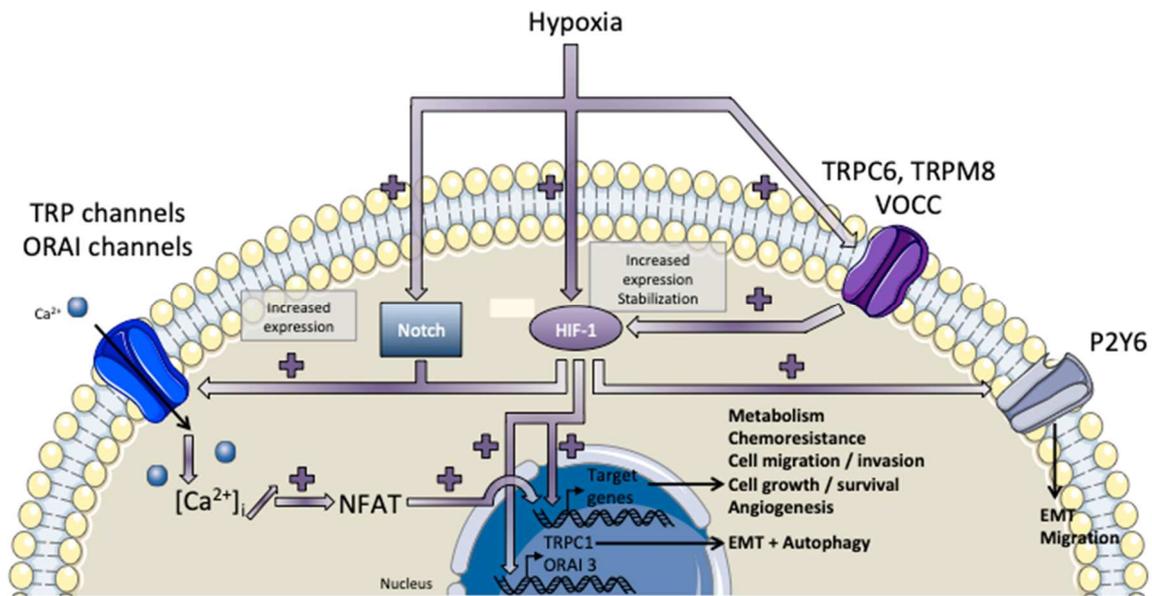


Figure 2. Involvement of Ca^{2+} channels in hypoxia conditions (in tumour or stellate cells)

Hypoxia triggers Notch or HIF-1 signaling pathway to increase the protein expression or the transcription of Ca^{2+} channels. Consequently, intracellular Ca^{2+} concentration increases and induces the transactivation of NFAT, which promotes the transcription of target genes involved in cell migration, invasion or neo-angiogenesis. HIF-1 could also promote the transcription of ORAI3 or TRPC1 channels. The latter will participate to the EMT or the autophagy processes. (HIF-1: Hypoxia Inducible Factor; NFAT: Nuclear Factor of Activated T-cell; EMT: Epithelial-to-Mesenchymal Transition).

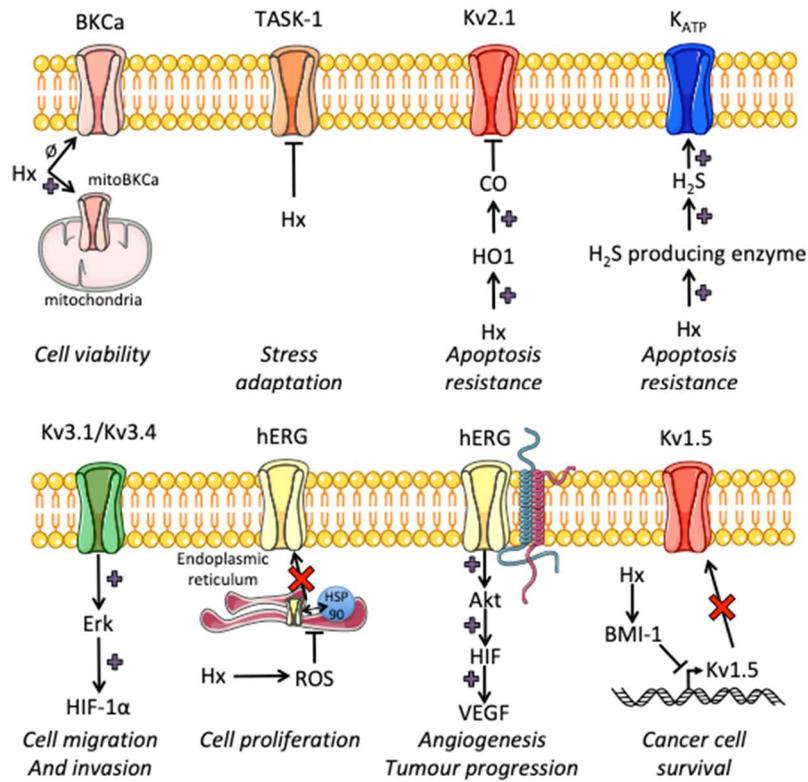


Figure 3. K⁺ channels signaling pathways in hypoxia conditions

Summary of the cell mechanisms reported in the literature to explain the effect of hypoxia involving K⁺ channels. (Hx: Hypoxia; mitoBKCa: mitochondrial BKCa; CO: carbon monoxide; HO1: Heme Oxygenase 1; H₂S: Hydrogen Sulfide; Erk: Extracellular signal-Regulated Kinase; HIF: Hypoxia Inducible Factor; ROS: reactive Oxygen Species; Akt: Akt Serine/Threonine Kinase 1 or Protein Kinase B; VEGF: Vascular Endothelial Growth Factor; BMI-1: polycomb complex protein BMI-1 or RING finger protein 51).