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Review

Ca^{2+} signaling and hypoxia/acidic tumour microenvironment interplay in tumour progression

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Abstract: Solid tumours are characterized by an altered microenvironment (TME) from the physicochemical point of view, displaying a highly hypoxic and acidic interstitial fluid. Hypoxia results from uncontrolled proliferation, aberrant vascularization and altered cancer cell metabolism. Tumour cellular apparatus adapt to hypoxia by altering its metabolism and behaviour, increasing its migratory and metastatic abilities by acquisition of a mesenchymal phenotype and selection of aggressive tumour cell clones. Extracellular acidosis is considered a cancer hallmark, acting as a driver of cancer aggressiveness by promoting tumour metastasis and chemoresistance by selecting for more aggressive cell phenotypes, although the underlying mechanism is still not clear. In this context, Ca^{2+} channels represent good target candidates due to their ability to integrate signals from the TME. Ca^{2+} channels are pH and hypoxia sensors and alterations in Ca^{2+} homeostasis in cancer progression and vascularization have been extensively reported. The present review will focus on Ca^{2+} permeable ion channels, with a major focus on TRP, SOC and PIEZO channels, that are modulated by tumour hypoxia and acidosis as well as the role of the resulted altered Ca^{2+} signals on cancer progression hallmarks. A deeper comprehension of the Ca^{2+} signaling and acidic pH/hypoxia interplay will break new ground for the discovery of alternative and attractive therapeutics targets.

Keywords: Ca^{2+} signaling; TRP channels; SOC channels; PIEZO channels tumour acidic microenvironment; hypoxia; tumour progression.

1. Introduction

1.1. Cancer microenvironment: focus on tumour acidic pHe and hypoxia

Tumours are characterized by a dynamic microenvironment constituted by a variety of different non-cellular components, such as the extracellular matrix components (ECM), circulating free DNA, and cell components, such as aberrant blood vessels, immune cells, tumour-associated fibroblasts (TAFs), endothelial cells, macrophages, pericytes, among others. Together with chemical and physical cues (hypoxia, tumour acidosis, high tumour interstitial stiffness), they originate a peculiar chemical and physical environment, in which interactions between these cues and the cellular and non-cellular components of the tumour microenvironment can assist cancer progression[1].

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A common feature of almost all advanced solid cancers is the presence of transient or permanent acidic and hypoxic tumour regions. In fact, the tumour microenvironment is a direct outcome of the rearrangement of the metabolic pathways of cancer cells, supporting the uncontrolled proliferation of cancer cells. This leads to a significant increase of cancer cells' anabolic activity and a reduction of the catabolic one, promoting the synthesis of amino acids, nucleotides, and lipids to back their growth. According to Otto Warburg work[2], cancer cells are characterized by an enhanced glycolytic breakdown of glucose to pyruvate and consequent NADPH and ATP production respect to healthy cells, even in presence of oxygen and even considering the lower energy yield of pyruvate fermentation compared to oxidative respiration. Nevertheless, it is important to underline that not all cancer cells are characterized by the Warburg effect, but it has been observed that cancer cells may have an opposite phenotype, with an increased mitochondrial oxidative activity[3]. The importance of mitochondrial activity in cancer cells is also explained by the oxidative phosphorylation increase observed when the Warburg effect is inhibited in cancer cells[3–5].

Fermentation of pyruvate resulting from glycolysis leads to a high production of lactic acid, acidifying the intracellular environment. Hydrolysis of ATP also determines the release of protons (H^+) in the intracellular space, contributing to its acidification[6–8]. Protonation has a severe negative impact on several enzymes and lipids leading to a potential risk of impinging several cellular processes including cell metabolism. It is therefore not surprising that cells use different systems to maintain the intracellular pH within the physiological range of about 7.2. In cancer cells, a slightly more alkaline intracellular pH (pH 7.4) has been observed, and evidences showed that this slight difference in pH_i between cancer cells and healthy cells promote some of the hallmarks of cancer, such as cell death escape and proliferation[9–12]. To reach this pH_i value, transformed cells have at their disposal an arsenal of overexpressed transporter proteins and pumps for protons and lactic acid extrusion to the extracellular milieu, resulting in its acidification. Examples of this transport system comprise monocarboxylate transporters (MCTs), major players in the transmembrane lactate trafficking, Na^+ /H^+ antiporters (NHE), vacuolar H^+ ATPases, and carbonic anhydrases (CAs), mainly CAIX and CAXII, which role in cancer progression is well documented[13–17]. Acidic interstitial fluids are not only given by the presence of lactic acid and protons, but also by CO_2, derived from cell respiration process in oxygenated areas. CO_2 can passively diffuse through plasma membrane (PM), or it can be reversibly hydrated to HCO_3^-, with the release of protons, by the transmembrane Carbonic Anhydrase IX (CAIX) exofacial site and released in the tumour microenvironment. Na^+/ HCO_3^- cotransporters (NBCs) in proximity of CAIX can mediate HCO_3^- influx for sustaining intracellular buffering, titrating cytosolic H^+ [18,19]. It has to be noticed that pH_i of cancer cells can drop significantly in the presence of a strong acidic pH_e, giving rise to a heterogeneous pH_i landscape (due to the TME), where cancer cells resident in acidic regions will present quite low pH_i, while cancer cells occupying moderate acidic pH_e areas will show a moderate alkaline pH_i[8]. It is therefore important to consider the tumour microenvironmental complexity to understand how cancer cells adapt to it, in order to possibly find new therapeutical

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targets. Tumour acidosis can be further supported by tumour-associated hypoxia, leading to higher glycolytic rates. Hypoxia occurs in the context of tumours vascularized by insufficient vessels and/or vessels characterised by a poor capacity to diffuse oxygen and nutrients and to remove the metabolic waste products, due to an altered process of angiogenesis, which leads to the formation of aberrant and dysfunctional vessels. Hypoxia is also due to an increased oxygen demand from highly proliferating tumours, leading to intratumor hypoxia heterogeneity, with subregions of the tumour characterized by different oxygen concentration and consumption, and the irregular exposure to oxygen fluctuations is associated with adaptive mechanisms set in motion by cancer cells in order to promote their survival in that hostile environment. Indeed, hypoxia adaptation is linked to increased genomic instability and tumorigenesis[20] and to more aggressive cancer phenotypes in terms of tumour growth, drug and cell death resistance, angiogenesis and enhanced metastasis [21].

Hypoxia adaptation processes are initiated by a series of transcription factors belonging to the Hypoxia-Inducible Factor family, in particular Hypoxia-Inducible Factor 1 (HIF-1), which determine a gene expression reprogramming that affects cancer cell metabolism and processes which sustain its progression. HIF-1 is a heterodimer protein constituted by HIF-1α and HIF-1β and this complex is not present in normoxic conditions. Although β subunit is constitutively expressed in all cells, HIF-1α subunit is present only at low levels in all cells' cytoplasm and this is not due to changes in its synthesis, but to the presence of two specific proline residues at positions 402 and 564 in the Oxygen-Dependent Degradation (ODD) domain in the α subunit. These residues are hydroxylated by prolyl hydroxylase protein (PHD) in presence of physiological oxygen levels and this modification targets the subunit to degradation via the ubiquitin-proteosome pathway[22]. In hypoxic conditions, hydroxylation of Pro402 and Pro564 doesn't take place due to PHD inhibition, allowing HIF-1α and HIF-1β to dimerize and translocate to the cell nucleus, where HIF-1 and p300 complex activate the transcription of target genes[22]. Beside low O_2 concentrations, also high intracellular lactate levels and specific growth factors or oncogenes can stabilise HIF-1α, leading to the activation of HIF-1α-target genes[23,24]."Hypoxia-adaptive" response genes include glucose transporters, such as GLUT1/3, enzymes involved in anaerobic glycolysis, such as lactate dehydrogenase-A (LDHA), aldolase (ALDA), phosphoglycerate kinase-1 (PGK1), enolase (ENOL) and phosphofructokinase-1 (PFK-1), pyruvate dehydrogenase kinase 1 (PDK1), with consequent suppression of Mitochondrial Oxidative Phosphorylation System (OXPHOS).

Hypoxia response is not limited to glycolytic flux, as it also enhances the expression of VEGF and other pro-angiogenic factors, and promotes tumour progression by inducing epithelial-mesenchymal transition (EMT) via different pathways[25], cell survival via autophagy[26] and metastasis, by sustaining the expression of different metalloproteases (MMPs), lysyl oxidase (LOX), connective tissue growth factor (CTGF) and CAIX, NHE1 and MCTs[21], which contribute to pH regulation and enhance the acidification of the tumour microenvironment.

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Hypoxia also plays a key role in chemoresistance, as reduced oxygen availability can affect not only drug delivery but also chemotherapeutics activity[27]. Moreover, tumour hypoxic core is occupied by cancer cells with an hypoxia-induced stem cell-like phenotype, characterized by cell cycle arrest in G1 phase and a quiescent state, representing a major problem for those chemotherapy agents which target rapidly proliferating cells[27–29]. Hypoxia also upregulates multidrug resistance genes[30–32]. All these mechanisms of cell adaptation to hypoxia determines the selection of highly aggressive clones, which pave the way for tumour expansion.

Besides being affected by the hypoxic tumour microenvironment, acidic pH_e has been observed to regulate HIF1α and HIF2α levels under normoxic conditions in glioma cells, promoting cancer stem cell maintenance[33], highlighting the feedback regulation and crosstalk between hypoxia and low pH_e. Similarly to hypoxia, acidic tumour microenvironment supports different hallmarks of cancer, such as drug resistance as previously described[34]. In addition, acidic TME plays an important role in immunoreactive processes and inflammation, by promoting the viability and fitness of pro-tumour M2 macrophages respect to anti-tumour M1 macrophages[35], by inhibiting T and NK cells activation and inducing immune escape[36,37], by inducing a phenotypic shift in macrophages towards a tumour-promoting phenotype[38] and by increasing the tumour-promoting functions of tumour-associated neutrophils[39]. Moreover, acidic pH_e fulfils its pro-tumour function through the enhancement of two other important hallmarks, cancer cell invasion and ability to metastasize[40]. Studies in breast cancer and colon cancer has demonstrated that invasive cell areas co-localize with acidic pH_e regions[41], while studies in melanoma cells have shown that acidic pH_e exposure increases their invasive abilities *in vitro* and the formation of pulmonary metastasis *in vivo* via a low pHe-promoted secretion of proteolytic enzymes and pro-angiogenic factors[42].

An explanatory example of the major role of acidosis in cancer progression is given by the unique pancreatic ductal adenocarcinoma (PDAC) microenvironment[43]. The pancreatic duct is a net acid-base transporting epithelium, in which ductal cells secret bicarbonate into the ductal lumen across the apical membrane. This transport is coupled to the extrusion of an equal amount of acid across the basolateral membrane, thereby physiological pancreatic interstitium is substantially acidic and epithelial cells are exposed to different extracellular pH (pH_e) values. This process is intermittent in healthy pancreas and associated with food intake. On the other hand, PDAC has been clearly associated with a hypoxic and acidic microenvironment with a dense desmoplastic stroma[43]. A challenging hypothesis is that, in combination with driver mutations, the alternating, but physiological, pH_e landscape in pancreas and the intrinsic ability of pancreatic epithelial cells to adapt to different pH conditions, may act as "preconditioning phenomenon" favouring the selection of specific cancer aggressive phenotypes which might promote PDAC arising and/or progression. In other words, once the specific mutations drive the ductal pancreatic cells transformation, cells would be already adapted and could even benefit of the adverse pH conditions and the combina-

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tions of these factors may increase cell fitness to survive and become strongly aggressive in hostile microenvironment[43].

In this context, it would be important to study the "transportome" alterations that are linked to pH_e or pH_i alterations as possible targets for therapies. Indeed, many of cancer hallmarks, such as cell proliferation, cell migration, invasion, apoptosis resistance, are driven by altered expression/regulation of ion transport proteins or ion channels including acid base transporters and pH sensitive channels, in particular Ca^{2+}- and pH sensitive ion channels[43,44].

1.2. Calcium signaling

Among different ions present in the intra and extracellular environments, Ca^{2+} ions stand out for their functional importance as second messengers. Ca^{2+} ions have been observed to crosstalk with several cell signalling pathways by promoting different spatio-temporal Ca^{2+} patterns to selectively regulate innumerable physiological cell processes, ranging from cell differentiation, proliferation, migration, programmed cell death to gene transcription, among others [45–47]. Its key role in signal transduction translates in the necessity of a tight regulation of intracellular Ca^{2+} homeostasis, maintaining a low cytosolic free Ca^{2+} concentration (100 nM) respect to the extracellular milieu (> 1mM) through the orchestrated work of several proteins that constitute the so-called Ca^{2+} signalling toolkit, including pumps (Ca^{2+} ATPases PMCA, SERCA) exchangers (Na^+/Ca^{2+} exchanger NCLX in mitochondria and NCX at the plasma membrane) or uniporters (MCU in mitochondria) and PM and ER Ca^{2+}- permeable channels. Calcium signals are modulated in time and space and transduced by cells to activate a specific response. In fact, to accomplish all the different cellular outcomes, calcium signals differ from each other in terms of amplitude, frequency, duration, and location [48,49].

Giving that tumour acidosis and hypoxia might support cancer progression, acting as drivers of its aggressiveness, and that Ca^{2+} signals dialogue with many tumour microenvironmental factors, such as pH and hypoxia, and Ca^{2+}-dependent signalling is known to be one of the major pathways leading to acquisition of cancer aggressive phenotypes[48,50], Ca^{2+} signalling may represent one communication mean between TME and cancer cells, where hypoxia and pH_e-sensitive members of calciosome (like Ca^{2+} permeable channels) might be affected by tumour acidosis and hypoxia and used to induce specific cancer phenotypes. Thus, acidic and hypoxic TME and Ca^{2+} signalling may work in synergy for the acquisition of aggressive cancer phenotypes. For this reason, a better understanding of the interplay between these players and the remodelling of Ca^{2+} signals induced by tumour acidic pHe and hypoxia and translated to the cancer cells through the activity of Ca^{2+}-permeable ion channels and pumps, may help to provide further comprehension of the mechanisms of cancer progression and novel putative therapeutic approaches. In the present review we will indeed present an updated view of recent literature on the role of Piezo channels, Transient Receptor Potential Ca^{2+}-permeable ions channels (TRPs) and the so-called Store-operated Ca^{2+} channels (SOCs), which activity and signalling transduction are directly affected by two features

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of the tumour microenvironment: hypoxia and acidosis. We will moreover illustrate the Ca^{2+} signaling pathways that may represent potential targets for cancer therapy.

Ion channel	Cell type	Methodology	Hypoxia technique, acidic pH value and treatment time	Effect of low pH/hypoxia on channel's activity/expression	Effect of low pH/hypoxia on Ca^{2+} signals	Cellular function	Ref.

2. Hypoxia and acidic pH_e-dependent regulation of Ca^{2+}-permeable ion channels in normal and cancer cells

Hypoxia and acidic pH_e regulate the expression and/or activity of several Ca^{2+}-permeable channels, which are linked to tumour aggressiveness. Throughout the years, several publications have revealed the role of these two major players of tumour microenvironment, revealing a marked sensitivity to oxygen and pH_e of most TRPs, SOCs and Piezo channels, which affects their functionality in different tissues. A detailed description of the updated literature will be the presented in this section and in Table 1.

As summarized in Table 1, the effects of tumour hypoxia and acidosis vary significantly between the different calcium-permeable channels, and the information in the literature regarding certain channels is sometimes contradictory or limited to normal cells.

Table 1.Ca^{2+}-permeable ion channels regulation by hypoxia and acidic pH.

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Piezo1	Piezo1-transiently transfected HEK293 cells	Patch clamp, Mn2+ quenching assay	pHe 6.3 - 6.7, acute treatment	Stabilization of inactivated state, both acidic pHi and pHe inhibit channel's activity	Decreased Ca^{2+} influx	Not assessed	[58]
	Murine pancreatic stellate cells (mPSCs)	Mn2+ quenching assay mPSCs spheroids viability and apoptosis assay	pHe 6.6 and pHi 6.77 (obtained by 30 mM propionate) in acute treatment for Mn^{2+} quenching assay, while 24h long treatment for spheroid histology	Acidic pHe do not modify Ca2+ influx, while intracellular acidification inhibits channel's activity	Acidic pHe do not modify Ca^{2+} influx, while intracellular acidification decreased Ca^{2+} influx	Acidic pHe (6.6) impairs PSCs spheroid's integrity and viability, inducing cell apoptosis	[60]
TRPM2	Inducible TRPM2-overexpressing HEK293	Patch clamp	External solution with pH 5-8 superfused for 200 seconds. Internal solution with pH 6 superfused for 100 seconds; External solution with pH 3.5-6.5 in acute treatment or more prolonged periods (≥2 minutes)	Extracellular acidification inactivates the channel in a voltage dependent manner and $[H^+]$-dependent manner, decreasing single-channel conductance. Intracellular acidification induces channel closure	Not assessed. But recovery from acidic pH-induced inactivation require external Ca^{2+} ions	Not assessed	[66]-[69]
	Human neutrophils	Patch clamp	External solution with pH 5 in acute treatment	External acidification negatively affects open probability and single-channel conductance, inducing channel closure	Not assessed	Not assessed	[66]
TRPM6	Pig isolated ventricular myocytes	Patch clamp	External solution with pH 5.5 and pH 6.5, ~5-10 minutes exposition	External acidification decreases channel's current amplitude in a pHe-dependent and voltage-independent manner. The inhibitory effect of acidic pHe is preventing by increasing intracellular pH buffering capacity	Not assessed	Not assessed	[84]
	TRPM6-overexpressing HEK293 cells	Patch clamp	External solution with pH 3-6, ~10 seconds-long exposition	External acidification increases channel's current amplitude in a pHe-dependent manner	Not assessed	Not assessed	[86]

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TRPM7	RBL-2H3 cells	Patch clamp	Acidification of intracellular side of membrane with ~200-seconds long t 4-40 mM acetate treatment	40 mM external acetate completely or pre-incubation in 40 mM acetate solution inhibit TRPM7 current in a reversible manner	Not assessed	Not assessed	[82]
	TRPM7-overexpressing Chinese Hamster Ovary (CHO-K1) cells	Patch clamp	Internal and external solution at pH 5.6 and variable exposition (~200-500 seconds)	Internal and external acidification abolish channels' current	Not assessed	Not assessed	[82]
	TRPM7-overexpressing HEK293 cells	Patch clamp	Internal solution at pH 6.1 and ~10 minutes exposition	Internal acidification decreases TRPM7 currents' density	Not assessed	Not assessed	[81]
	Mouse hippocampal neurons	Patch clamp	External solution with pH 6.5, 2 minutes exposition	Extracellular acidification slows down channel's activation in a voltage-independent way	Not assessed	Not assessed	[83]
	TRPM7-overexpressing HEK293T cells	Patch clamp	External solution with pH 4 and pH 6, acute treatment	External acidification increases channel's current amplitude in a pHe-dependent manner	Not assessed	Not assessed	[86]
	TRPM7-overexpressing HEK293T cells	Patch clamp	External solution with pH 3-7, ~50-seconds-long exposition	External acidification determines a significant increase of TRPM7 inward current in a [H+] concentration-dependent manner, with maximum effect at pHe 3, by potentiating	Not assessed	Not assessed	[85]
	Pig isolated ventricular myocytes	Patch clamp	External solution with pH 5.5 and pH 6.5, ~5-10 minutes exposition	External acidification decreases channel's current amplitude in a pHe-dependent and voltage-independent manner. The inhibitory effect of acidic pHe is prevented increasing intracellular pH buffering capacity	Not assessed	Not assessed	[84]
	Rat basophilic leukemia cells (RBL)	Patch clamp	External solution with pH 5.5, pH 6 and pH 6.5, ~1-minute-long exposition	External acidification decreases channel's current amplitude in a pHe-dependent manner	Not assessed	Not assessed	[84]
	HeLa cells	Patch clamp Cell death assays (fluometric analysis	External solution with pH 4 and pH 6,	External acidification increases channel's current	Not assessed	Acidosis promotes HeLa necrotic cell death	[87]

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		of caspase 3/7 activation, electronic sizing of cell volume, and triple staining with Hoechst/acridine orange and propidium iodide assay.	acute treatment for patch clamp experiments. 1h-long treatment with acidic pHe (4 and 6) for cell death assays	amplitude in a pHe-dependent manner			
	Human atrial cardiomyocytes	Patch clamp	External solution with pH 4- 6, acute treatment	External acidification increases channel's current amplitude in presence of divalent cations in the extracellular milieu	Not assessed	Not assessed	[88]
	TRPM7-overexpressing HEK293T cells Cortical neurons	Ca2+ imaging Patch clamp PI cell death assay	Hypoxia induced by anaerobic chamber containing <0.2% O2 atmosphere for 1, 1.5 and 2h.	Hypoxia induces TRPM7 channel activation	Anoxia increases Ca2+ entry	Hypoxia-activated TRPM7 mediated-Ca2+ entry determines cell death in cortical neurons	[89]
TRPV1	TRPV1-expressing HEK293 cells	Patch clamp	Acidic solution with pH 5.5 applied intracellularly for ~50 seconds;	Acid treatment does not activate the channel in inside-out patches but potentiates 2-APB-evoked currents from the cytoplasmic side	Not assessed	Not assessed	[97]
	hTRPV1-transfected HEK293t cells	Calcium imaging	External solution with pH 4.3 and pH 6.1, ~4 minutes-long exposition	Acidic pH activates TRPV1 channel	pHe 6.1 determines larger Ca^{2+} transients respect to pHe 4.3 in physiological extracellular Ca^{2+} concentration, while, in presence of low extracellular Ca^{2+} concentration, cells exposed to pHe 6.1 show a reduced Ca^{2+} entry respect to pHe 4.3 exposition.	Not assessed	[98]
	Defolliculated *Xenopus laevis* oocytes, TRPV1-expressing HEK293 cells	Patch clamp	Extracellular solution with pH 6.4, cells pre-treated with acid bath solution for 2 min	Acidic pHe potentiates heat-evoked TRPV1 current in oocytes; potentiation of capsaicin and heat-evoked TRPV1 currents in HEK293 cells	Not assessed	Not assessed	[95]
	Primary human	Cell viability assay,	24h-long ex-	Acidic pHe acti-	Not assessed	Acidic pHe affects	[100

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	adult dermal lymphatic endothelial cell (HDLECs)	cell invasion assay, *in vitro* tube formation assay, transwell cell migration assay	position to pHe 6.4. 6h-long exposition for *in vitro* tube formation assay	vates TRPV1 channel		HDLECs morphology, increasing their migration and invasive abilities, proliferation and promoting lymphangiogenesis via acidosis-induced TRPV1 activation	]
TRPV2	TRPV2-expressing HEK293 cells	Patch clamp	Acute administration of extracellular solution with pHe 5.5 and 6	Extracellular acidosis potentiates the response of TRPV2 to 2-APB (and analogs) from the cytosolic side, while intracellular acidification and low pHe alone are not able to elicit any detectable current	Not assessed	Not assessed	[97]
TRPV3	TRPV3-expressing HEK293 cells	Patch clamp, calcium imaging	Acute administration of extracellular solution with pHe 5.5 and 6	Extracellular acidosis potentiates the response of TRPV3 to 2-APB (and analogs) from the cytosolic side. Intracellular acidification activates the channel, eliciting small but detectable currents	Extracellular acidosis increases Ca^{2+} entry following 2-APB stimulation	Not assessed	[97]
	TRPV3-expressing HEK293 cells	Patch clamp cell death assay (PI staining assay)	Intracellular administration of acidic solution with pHe 5.5 and glycolic acid. Extracellular solution with pH 5.5. Intracellular solution with pH 5.5-7.	Glycolic acid-induced intracellular proton release in presence of acidic solution activates the channel in a reversible way. Extracellular acidification does not activate TRPV3, while intracellular acidification alone activates the channel in a pH-dependent manner	Not assessed	Glycolic acid-induced acidification induces cell toxicity and cell death	[104]
	Human keratinocytes cells (HaCaT)	Patch clamp, cell death assay (PI staining assay)	Intracellular administration of acidic solution with pHe 5.5 and glycolic acid	Glycolic acid-induced intracellular proton release in presence of acidic solution potentiates the channel's response to 2-APB in a reversible	Not assessed	Glycolic acid-induced acidification induces cell toxicity and cell death	[104]
	mTRPV3-transiently transfected HEK293 cells	Patch clamp, calcium imaging	External solution with pHe 5.5 and 6.5, 1-2 minutes-long treatment.	Extracellular acidosis potentiates the response of TRPV2 to 2-APB due to proton	Extracellular acidosis increases Ca^{2+} entry following 2-APB	Not assessed	[103]

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			Extracellular solution with pH 5.6 for calcium imaging experiments, with a 3 minutes-long exposition.	modification of the compound but decreases channel's response to carvacrol. Intracellular acidification facilitates sensitization of TRPV3.	stimulation		
TRPV4	Chinese hamster ovary cells	Patch clamp	External solution with pHe 4, 5.5 and 6, acute treatment	Extracellular acidosis activates the channel in a pHe-dependent manner	Not assessed	Not assessed	[114]
	mTRPV4-overexpressing primary cultured mouse esophageal epithelial cells	Ca^{2+} imaging	External solution with pHe 5, acute treatment	Not assessed	Extracellular acidic pH decreases Ca^{2+} entry, lowering cytosolic Ca^{2+} concentration	Not assessed	[115]
TRPV6	Jurkat cells	Patch clamp	External solution with pH 6, acute treatment	Extracellular acidosis suppresses TRPV6-mediated currents	Extracellular acidic pH reduces Ca^{2+} entry, lowering cytosolic Ca^{2+} concentration	Not assessed	[123]
TRPA1	HEK-293t cells expressing hTRPA1, mTRPA1, or rTRPA1	Patch clamp, calcium imaging	Acidic solutions with pH 7.0, 6.4, 6.0, and 5.4, 30 seconds-long treatment in calcium imaging experiments	Extracellular acidosis activates inward currents via hTRPA1 and potentiates acrolein-evoked currents of hTRPA1 in a pHe-dependent and reversible manner, while failed to activate mouse and rodent TRPA1.	Extracellular acidosis increases Ca^{2+} entry in hTRPA1, no effect on mTRPA1 and rTRPA1.	Not assessed	[134]
	Several breast and lung cancer cell lines	Calcium imaging Cell viability and apoptosis assay via PI and Annexin IV staining	Treatment with 10 μM H_2O_2 for 15 minutes for calcium measurements, 1, 20 and 100 μM for 72-96h-long exposition for cell viability and cell death assays	H_2O_2 treatment activates TRPM7 channel	H_2O_2 treatment increases TRPM7-mediated calcium entry	TRPM7-mediated calcium entry promotes cell survival by upregulating anti-apoptotic pathways	[128]
	DRG neurons derived from TRPV1/TRPA1-/- mice and overexpression hTRPA1	Calcium imaging	Acidic solutions with pH 5, 60 seconds-long treatment	Not assessed	Acidic pHe induces Ca^{2+} entry	Not assessed	[134]
	Neuroblastoma ND7/23 cells expressing hTRPA1	Patch clamp	Acidic solutions with pH 5, acute treatment	Acidic pH activates hTRPA1	Not assessed	Not assessed	[134]

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	Oligodendrocytes	Calcium imaging	Ischemia inducing solution	Not assessed	Ischemia-induced intracellular acidosis promotes Ca^{2+} entry via TRPA1	Ischemia-induced intracellular acidosis and consequent Ca2+ entry via TRPA1 mediate myelin damage	[135]
TRPC1	U-87 MG glioma cells	qPCR, western blot	Hypoxia induced by exposition to 1% O_2	Not assessed	Not assessed	TRPC1 is involved in hypoxia-induced VEGF gene and protein expression	[142]
	MDA-MB-468 breast cancer cells	qPCR, calcium imaging	Hypoxia induced by exposition to 1% O_2 for 24h	Hypoxia upregulates TRPC1 via HIF1α	siTRPC1 reduces non-stimulated Ca^{2+} entry and increases Store-Operated Ca^{2+} entry in hypoxic conditions	TRPC1 overexpression promotes Snail EMT marker upregulation and decrease of claudin-4 epithelial marker in hypoxic conditions. TRPC1 regulates HIF-1α protein levels via Akt-dependent pathway and promotes hypoxia-induced STAT3 and EGFR phosphorylation. RPC1 also regulates hypoxia-induced LC3BII levels via effects on EGFR.	[144]
TRPC5	MCF-7/WT and adriamycin-treated (MCF-7/ADM) human breast cancer cells	Western Blot, Immunofluorescence,	Not assessed	Not assessed	Not assessed	TRPC5 promotes HIF-1alpha translocation to the nucleus and HIF-1alpha-mediated VEGF expression, boosting tumor angiogenesis	[145]
	SW620 colon cancer cells	Western blot, transwell invasion and migration assay, MTT proliferation assay	Not assessed	Not assessed	Not assessed	TRPC5 activates HIF-1alpha-Twist signaling to induce EMT, supporting colon cancer cells' migration, invasion, and proliferation	[146]
	TRPC5-transiently transfected HEK293 cells	Patch Clamp	External acidic solution with pH 4.2, 5.5, 6.5, 7, ~100 seconds-long treatment	G protein-activated and spontaneous currents are potentiated by extracellular acidic pH by increasing the channel open probability, with a maximum effect at ~pH 6.5, while more acidic values inhibit the channel.	Not assessed	Not assessed	[147]
TRPC4	TRPC4-	Patch Clamp	External acidic	G protein-	Not assessed	Not assessed	[147

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	transiently transfected HEK293 cells		solution with pH 4.2, 5.5, 6.5, 7, ~100 seconds-long treatment	activated currents are potentiated by extracellular acidic pH, with a maximum effect at ~pH 6.5 and complete inhibition at pHe 5.5			]
	mTRPC4-stably transfected HEK293 cells	Patch Clamp	External acidic solution with pH 6.8	Low pHi (6.75–6.25) accelerated $G_{i/o}$-mediated TRPC4 activation, and this requires elevations in intracellular calcium concentration. Intracellular protons inhibit Englerin A-mediated TRPC4 activation	Not assessed	Not assessed	[157]
TRPC6	TRPC6-transiently transfected HEK293 cells	Patch Clamp	External acidic solution with pH 4.2, 5.5, 6.5, 7, ~100 seconds-long treatment	Acidic pHe inhibits channel's inward and outward currents starting from pHe 6.5 and the inhibition is potentiated by more acidic pHe values.	Not assessed	Not assessed	[147]
	Murine pancreatic stellate cells (mPSCs)	Time-lapse single cell random migration assay Bead-based cytokine assay qPCR Western Blot Ca2+ signals quantification by Mn^{2+} quench technique	24 h incubation in hypoxic conditions (1% O_2, 5%CO_2, and 94% N_2) or chemically-induced hypoxia by pretreatment with 0.5 mmol/l DMOG	Hypoxic conditions enhance TRPC6 expression and activates the channel	Hypoxia stimulates Ca^{2+} influx mediated by TRPC6 channels	Hypoxia-induced TRPC6 activation enhances mPSCs migration via secretion of pro-migratory factors	[158]
	lx-2 human hepatic stellate cells (HSCs)	Calcium imaging qPCR Western Blot	Hypoxia induced by 100 µmol/L $CoCl_2$ treatment	Hypoxic conditions enhance TRPC6 expression and activates the channel	Hypoxia stimulates Ca^{2+} influx mediated by TRPC6 channels	Hypoxia-induced TRPC6 activation and consequent calcium entry promotes the synthesis of ECM proteins, which facilitate the fibrotic activation of HSCs	[161]
	Huh7 and HepG2 hepatocellular carcinoma cells (HCCs)	Confocal Calcium imaging Western Blot	Hypoxia induced by cell incubation in a low oxygen atmosphere with 1% O_2, 5%CO_2, and 94% N_2 for 6 h	Hypoxic conditions activates the channel	Hypoxia promotes calcium influx	Hypoxia-induced TRPC6- mediated calcium entry promotes HCCs drug resistance via STAT3 pathway	[159]
	U373MG and HMEC-1 glioblastoma cell lines	qPCR Western Blot Calcium imaging Proliferation assay Matrigel invasion assay	Hypoxia induced by 100 µmol/L $CoCl_2$ treatment	Hypoxia enhances TRPC6 expression via Notch pathway	Hypoxia stimulates Ca^{2+} influx mediated by TRPC6 channels	Hypoxia-induced TRPC6- mediated calcium entry promotes HCCs proliferation, colony formation and	[160]

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		Endothelial cell tube formation assay				invasion via NFAT pathway	
ORAI1/STIM 1	Human macrophages	Patch clamp	External acidic solution with pH 6 and 8, ~200 seconds-long treatment	Extracellular acidosis inhibits ORAI1 channel in a pHe-dependent and reversible manner	Not assessed	Not assessed	[178]
	H4IIE rat liver cells overexpressing ORAI1 and STIM1	Patch clamp	External acidic solutions with pH 5.1 and 5.9	ORAI1 and STIM1-mediated I_{CRAC} were inhibited by acidic pHe, with maximal effect at pHe 5.5	Not assessed	Not assessed	[179]
	RBL2H3 mast cell line, Jurkat T lymphocytes and heterologous ORAI1-2-3/STIM expressing HEK293 cells	Patch clamp	External and intracellular acidic solutions with pH 6 and 6.6	External and internal acidification inhibits IP3-induced I_{CRAC} in RBL2H3 mast cell line, Jurkat T lymphocytes and in heterologous ORAI/STIM-mediated ICRAC in HEK293 cells in a reversible manner	Not assessed	Not assessed	[180]
	ORAI1/STIM1-transiently transfected HEK293 cells	Patch Clamp	External acidic solution with pH 5.5	Acidic pHe inhibits ORAI1-2-3/STIM1 current amplitude in a reversible and pH-dependent manner, with a maximal effect at pHe 4.5	Not assessed	Not assessed	[181]
	ORAI1/STIM1-transiently transfected HEK293 cells	Patch Clamp	Intracellular acidic solution with pH 6.3	Intracellular acidosis inhibits ORAI1/STIM1 current, regulating the amplitude of the current and the Ca^{2+}-dependent gating of the CRAC channels	Not assessed	Not assessed	[182]
	Primary Aortic Smooth Muscle Cells and HEK293 cells transfected with ORAI1 and STIM1	Patch Clamp Calcium imaging	Hypoxia was induced with 3 methods: 1) sodium dithionite ($Na_2S_2O_4$) treatment to 1 mM final concentration, pH adjustment to pH 7.4 and bubbling with 100% N_2. 2) cell culture media with 30 minutes- long bubbling with 100% N_2. 3) cell culture	Intracellular acidification induced by hypoxia in HEK293 cells leads to inhibition of SOCE by disrupting the electrostatic ORAI1/STIM1 binding and closing ORAI1 channel.	Hypoxia-induced intracellular acidification reduces SOCE in Primary Aortic Smooth Muscle Cells and HEK293 cells transfected with ORAI1 and STIM1	Not assessed	[184]

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			media with 30 minutes- long bubbling with 3% O_2				
	SH-SY5Y human neuroblastoma cells	Ca^{2+} signals quantification by Mn^{2+} quench technique	External acidic solution with pH 6.8 and 7 and 7.2. Different treatment time, ranging from ~3-4 minutes to ~8 minutes for carbachol-mediated Ca^{2+} entry and ~7 minutes for thapsigargin-mediated Ca^{2+} entry	Not assessed	Tumor acidic pHe inhibits carbachol- and thapsigargin-mediated Ca2+ entry in a reversible manner, while intracellular acidification or alkalinization leads to no effects in carbachol-mediated Ca2+ entry	Not assessed	[185]
	A549 non-small cell lung cancer cells	Western Blot qPCR BrdU cell proliferation assay Calcium imaging Scrape-wound migration assay Matrigel transwell invasion assay	Hypoxia induced by Nicotine treatment (0, 0.1, 1, 10 and 100 μM) for 48h in 0.1% FBS medium. 1 μM Nicotine treatment for 48 h for Calcium imaging experiments.	Nicotine treatment-induced hypoxia determines ORAI1 overexpression at gene and protein levels	Nicotine treatment-induced hypoxia increases intracellular basal calcium levels and SOCE	Nicotine treatment-induced hypoxia increases A549 cells' proliferation and migration	[186]
	MDA-MB 231 and BT549 breast cancer cell lines and Human Microvascular Endothelial Cell line-1 (HMEC-1)	Western Blot qPCR Calcium imaging Migration assay (Wound healing and transwell migration assay) Matrigel transwell invasion assay Tube formation assay in vitro	Hypoxia induced by cell incubation in a low oxygen atmosphere with 1% O_2, 5%CO_2, and 94% N_2 for 0, 4, 8 and 12h for WB experiments, while for 16h for functional assays	Hypoxia promotes gene and protein upregulation via activation of Notch1 signaling	Hypoxia increases thapsigargin-induced SOCE, with consequent rise in cytosolic calcium entry	Hypoxia-induced ORAI1 overexpression and consequent increase in SOCE promotes NFAT4 activation and enhances neuroblastoma cells' migration, invasion and angiogenesis	[170]
	HCT-116 and SW480 human colon cancer cells and Human Microvascular Endothelial Cell line-1 (HMEC-1)	Western Blot qPCR Calcium imaging Transwell migration assay Matrigel transwell invasion assay Tube formation assay in vitro Cell attachment and detachment assays	Hypoxia induced by 100 μmol/L $CoCl_2$ treatment	Hypoxia promotes gene and protein upregulation via activation of Notch1 signaling	Hypoxia increases thapsigargin-induced SOCE	Hypoxia-induced ORAI1 overexpression and consequent increase in SOCE promotes NFATc3 activation and enhances neuroblastoma cells' migration, invasion and angiogenesis	[171]

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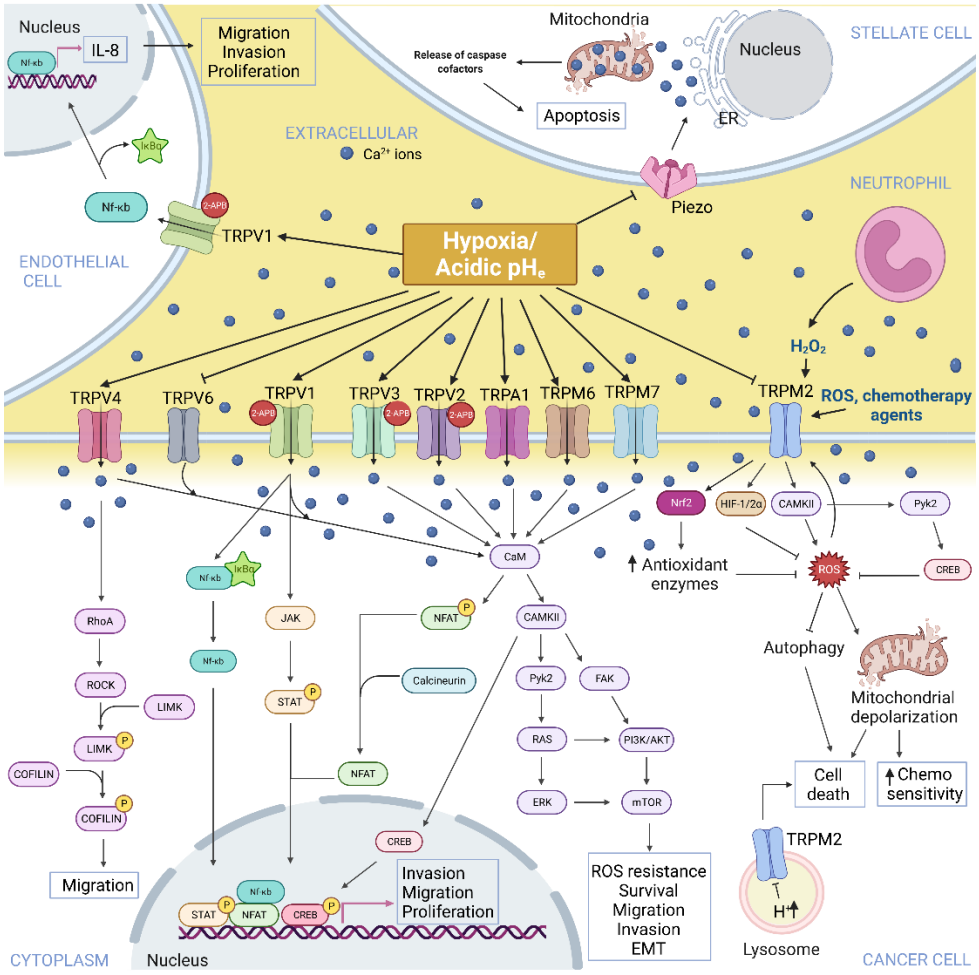

Figure 1. Overview of the Piezo-, TRPV-, TRPA1- and TRPM-mediated Ca^{2+}-dependent signaling pathways activated or inhibited by acidic pH_e and linked to tumour progression. TRPV (TRPV1-4, 6), TRPA1 and TRPM (TRPM6,7) expressed in cell cancer's plasma membrane are differentially regulated by acidic pH_e, being mostly activated by tumour acidosis and transducing its signals to activate Ca^{2+}-dependent downstream effectors, such as NF-κB, JAK/STAT, PI3K/AKT, NFAT, ERK and LIMK. TRPA1 is also activated by hypoxiaThese effectors promote tumour cell migration, invasion, proliferation, survival, mesenchymal phenotype and chemoresistance. TRPV6 channels' activity is inhibited by tumour acidosis, as TRPM2, which inhibition avoids induction of cancer cell death and reduces chemosensitivity. Piezo channels embedded in stellate cells' plasma membrane are inhibited by acidic pH_e, promoting stellate cells' survival. TRPV1 activation in lymphatic endothelial cells promotes activation of NF-kB and upregulation of IL-8, a lymphangiogenic factor. CaM, calmodulin; CAMKII, Ca^{2+}/calmodulin-dependent protein kinase II; Pyk2, protein tyrosine kinase 2; RAS, Rat sarcoma virus; ERK, extracellular signal-regulated kinase; FAK, Focal Adhesion Kinase; PI3K, phosphoinositide 3-kinase; AKT, protein kinase B; mTOR, mammalian target of rapamycin; NF-κB, nuclear factor-κB; JAK, Janus kinases; STAT, signal transducer and activator of transcription; NFAT, Nuclear factor of activated T-cells; RhoA, Ras homolog family member A; ROCK, Rho-associated protein kinase; LIMK, LIM domain kinase; CREB, C-AMP Response Element-binding protein. Created with BioRender.com.

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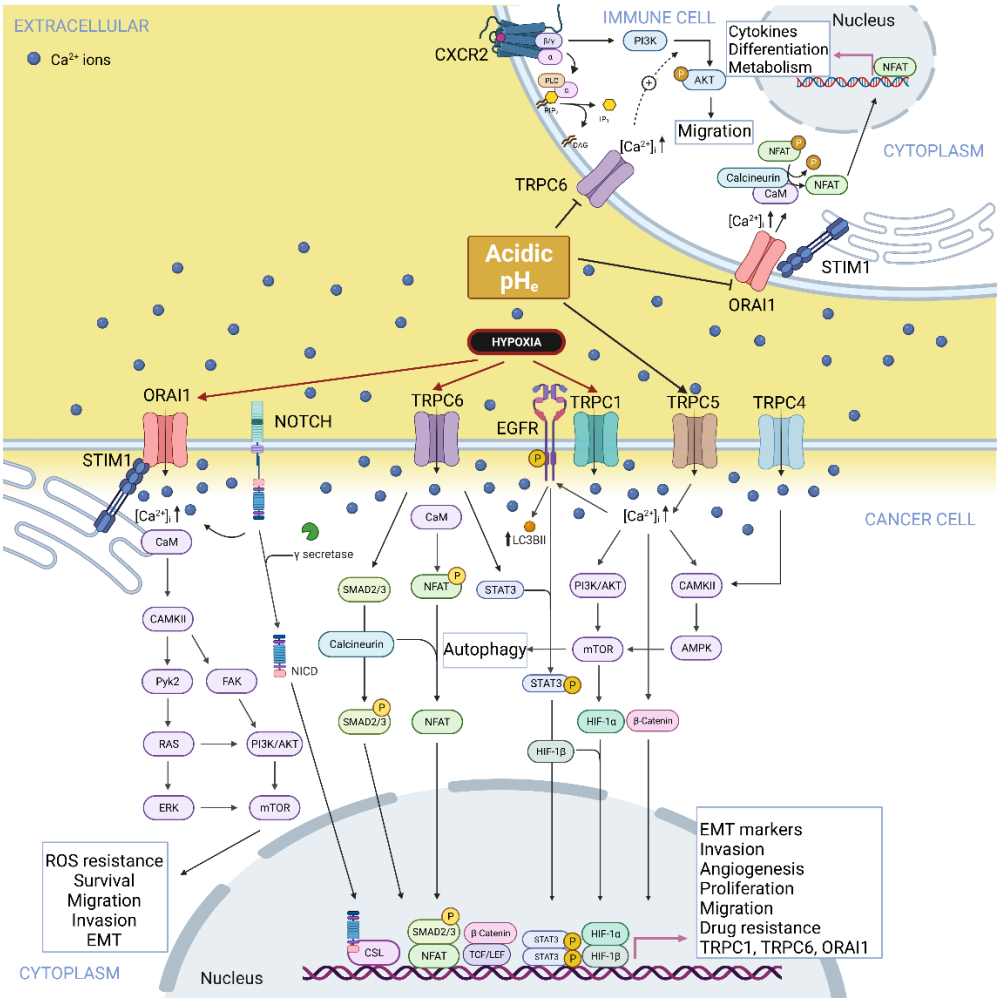

Figure 2. Overview of the TRPC and SOCs-mediated Ca^{2+}-dependent signaling pathways inhibited or activated by hypoxia and acidic pH_e and linked to tumour progression. TRPC (TRPC1, 4, 5) expressed in cell cancer's plasma membrane are all activated by acidic pH_e or hypoxia, transducing their signals to activate Ca^{2+}-dependent downstream effectors, such as SMAD2/3, NFAT, STAT3, HIF1, AMPK and β-catenin. These effectors promote tumour cell migration, angiogenesis, invasion, proliferation, mesenchymal phenotype and chemoresistance and the expression of TRPC1, via EGFR activation, and TRPC6 via Notch1 signaling pathway, in a mechanism of positive feedback regulation for both TRPC1 and TRPC6 channels. Immune cells expressing TRPC6 channels on plasma membrane show TRPC6's activity that is inhibited by acidic pH_e, reducing their migration. ORAI1 channels function in immune cells is also negatively affected by acidic pH_e, impairing different processes needed for immune cells' anti-tumour activity. Hypoxia promotes both ORAI1 expression, via Notch signaling pathway, and activation, leading to increased ROS resistance, migration, invasion, EMT and cell survival. CaM, calmodulin; CAMKII, Ca^{2+}/calmodulin-dependent protein kinase II; Pyk2, protein tyrosine kinase 2; RAS, Rat sarcoma virus; ERK, extracellular signal-regulated kinase; FAK, Focal Adhesion Kinase; PI3K, phosphoinositide 3-kinase; AKT, protein kinase B; mTOR, mammalian target of rapamycin; NICD, Notch intracellular domain; CSL, CBF1, Suppressor of Hairless, Lag-1; NFAT, Nuclear factor of activat-

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ed T-cells; STAT, Signal Transducers and Activators of Transcription; EGFR, Epidermal Growth Factor Receptor; HIF-1, Hypoxia Inducible Factor 1. Created with BioRender.com.

2.1. Piezo Channels

Piezo proteins are non-selective cations channels that were identified over a decade ago, when Patapoutian and colleagues showed for the first time that Piezo1 and Piezo2 are mechanically activated ion channels in mammals[51], a discovery that paved the way for various works that further clarified the role of these channels not only in the transduction of mechanical signals, but also in other physiological processes, and culminating in the 2021 Nobel Prize in Physiology or Medicine for David Julius and Ardem Patapoutian "for their discoveries of receptors for temperature and touch". Piezo are non-selective cation channels, with a slight greater permeability to Ca^{2+}[51]. They play a key role in mechanotransduction, directly responding and integrating mechanical stimuli and forces from the cell environment into biological signals, leading to the activation of Ca^{2+}-dependent processes or cell depolarization in different organs[51]. Piezo channels are also involved in other physiological processes, exhaustively reviewed in [51].

The role of Piezo channels in cancer has been deepened in recent years, with several studies evidencing their importance in different types of cancer that originate from tissues subjected to mechanical stress[52]. Piezo1 and/or Piezo2 are overexpressed in several cancers of epithelial origin, where these channels act as oncogenes, enhancing carcinogenesis through different Ca^{2+}-dependent signaling pathways[53–57].

Besides being sensitive to mechanical stimulation, Piezo1 is also regulated by protons. The work of Bae C. and colleagues of 2015 demonstrated that conditions of acidosis (pH_e 6.3) inhibit Piezo1 by stabilizing its inactivated state[58]. Drop in extracellular pH and inactivation of Piezo1 might represent a protective mechanism in specific cell types, especially considering that low extracellular pH can promote the intracellular acidification and, therefore, the activation of specific signalling pathways that result in cell death[59] (Table 1). This notion is supported by the study of Kuntze A. et al. of 2020, which demonstrated that in PDAC extracellular acidosis-induced low intracellular pH (pH_i 6.7) of pancreatic stellate cells (PSCs) inhibits the activity of Piezo1, reducing the Ca^{2+} influx in PSCs (Fig. 1 and Table 1). In these conditions, Piezo1 activation with Yoda1 leads to a loss of PSCs spheroid integrity and increased fragmentation, resulting virtually by Ca^{2+} overload and its induction of cell death. Therefore, extracellular acidosis-mediated intracellular pH drop and inactivation of Piezo1 represent a protective mechanism for PSCs, in which Ca^{2+} fluxes are decreased, and apoptosis is avoided [60].

2.2. Transient Receptor Potential Channels

Transient receptor potential (TRP) ion channels are a family of 28 different proteins in humans, mostly permeable to Ca^{2+} ions, characterized by a polymodal activation, and

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whose altered expression and/or functionality has been linked to several cancer types [61]. Several TRP channels are sensitive to changes in intra- and extracellular pH (see Table 1), altering Ca^{2+} downstream signaling pathways as described in the following paragraphs.

2.2.1. TRP melastatin subfamily

TRPM2 is a non-selective cation channel localized at the plasma membrane and/or in lysosome compartments and permeable to Ca^{2+}, Mg^{2+} and monovalent cations. TRPM2 is activated by ADP-ribose (ADPR) as well as intracellular Ca^{2+} increase associated with oxidative stress and ROS production[62–65].

TRPM2's activity is regulated by both intra- and extracellular pH. The work of Starkus and colleagues demonstrated that extracellular acidic pH (IC_{50} pH= 6.5) inhibits both inward and outward TRPM2 currents in TRPM2-overexpressing HEK293 cells in a voltage-dependent manner, by affecting the single-channel conductance, most probably due to the interaction of protons with outer pore and competing for binding sites with extracellular Ca^{2+} ions, as these ions attenuate the inhibitory effect of pH_e on TRPM2[66]. These results were confirmed the same year by Du J. et al. on the same cell line, although assuming a non-proton permeation through the channel[67], and by Yang W. et al. in 2010[68], showing that low pH_e-mediated inhibition of TRPM2 might be induced by conformational changes following protons binding. Interestingly, extracellular acidic pH effects are species-dependent, with mTRPM2 channels showing less sensitivity to acidic pH_e compared to hTRPM2[69] (see also Table 1). Intracellular acidic pH has also a reversible inhibitory effect on the channel, inducing its closure, without affecting single-channel conductance[66] and probably by a mechanism of proton competition with the Ca^{2+} and ADPr binding site[67]. Du J. et al. identified D933 residue (at the linker between S4-S5) as important residue for proton sensitivity[67].

This channel is upregulated in several cancers with a pro-tumour effect via Ca^{2+}-dependent pathways[70–75]. This effect may be explained by the protective role promoted by TRPM2 activation that act as a ROS sensor and promotes in turn activation of transcription factors involved the increase level of antioxidant (i.e. HIF-1/2a; CREB; NrF2)[70] (Fig. 1). However, other studies correlate TRPM2 expression with a higher sensitivity to chemotherapy. Indeed, an anti-survival role was highlighted in breast and colon cancer, where TRPM2 activation by chemotherapy agents resulted in Ca^{2+} entry, intracellular Ca^{2+} overload and increased mitochondrial depolarisation, leading to cell death[76], and in prostate cancer, where H_2O_2-induced TRPM2 activation results in PC3 cells' death via Ca^{2+}-dependent inhibition of autophagy[77] (Fig. 1). Another important consideration is the key role of TRPM2 in neutrophil-mediated cytotoxicity. Neutrophils secrete H_2O_2, which activates TRPM2 expressed on cancer cells' surface. This activation leads to Ca^{2+} influx in cancer cells, resulting in intracellular overload and induction of cell death, as was demonstrated in breast cancer cells[78] (Fig.1). Therefore, despite the pro-proliferative role of TRPM2 in several cancer cell lines, inhibition of its activity by tumour extracellular acidic pH might result in cancer cells' protection from

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neutrophil cytotoxicity, with an overall major efficiency in dissemination. Beside this, TRPM2 is also localized in lysosomal membranes, where the highly acidic pH inside the compartment might prevent TRPM2 activation and Ca^{2+} release from lysosomes to the cytosol, which may trigger apoptosis[79] (Fig. 1). These works reinforce the concept that TRPM2 inhibition by acidic pHe represents a protective mechanism for cancer cells.

Other members of the TRP melastatin subfamily which activity is modulated by pH are **TRPM6** and **TRPM7**, both permeants mainly to Mg^{2+} ions and contributing to its homeostasis. These channels are also permeant to Ca^{2+} ions, increasing its intracellular concentration. TRPM7 has a unique structure as a "chanzyme" due to the presence of a kinase domain in its structure. TRPM6 has a tissue-specific expression and it is down-regulated in several cancer types, while TRPM7 is ubiquitously expressed and mostly upregulated in different malignancies, where it plays a key role in promoting different cancer hallmarks[80].

TRPM6 and TRPM7 channels' activity is modulated by both intra- and extracellular pH. For what concerns the effects of intracellular pH on TRPM7, the outward current density is decreased at low intracellular pH in HEK293 cells, with an IC_{50} of pH 6.32 and in the absence of Mg^{2+}[81]. This result was previously obtained also in RBL cells, where native TRPM7 currents were inhibited by intracellular acidification obtained by acetate treatment, and in TRPM7-overexpressing HEK293 cells in the same work [82]. Intracellular alkalinisation, induced by NH_4^+ extracellular application, determines the induction of native TRPM7 current and the enhancement of its activity in RBL cells[85] (Table 1).

Quite variable effects of acidic pH_e (< 6.0) have been reported on TRPM7 activity, some works demonstrating the TRPM7 currents inhibition by low pH_e [81–84], others the potentiation of TRPM7 inward current by strong acidic pHe in HEK293 cells[85,86] and HeLa human cervical cells[87] (Table 1). The discrepancy of pH_e modulation on TRPM7 described by these works could be explained taking into consideration the importance of Ca^{2+} and Mg^{2+} ions presence in the extracellular milieu. According to the work of Mačianskienė R. et al., both TRPM7 outward and inward currents, expressed by cardiomyocytes, are potentiated in acidic extracellular medium (pH< 6) containing divalent Ca^{2+} and Mg^{2+} ions, while the absence of these ions in the acidic extracellular medium resulted in the low pH_e-mediated inhibition of TRPM7 currents in a voltage-independent manner[88]. Indeed, the works showing an inhibitory effect of extracellular acidification were performed in divalent-free conditions, masking the effects of Ca^{2+} and Mg^{2+} in blocking the low pHe-inhibitory effect on TRPM7. It is hypothesized that protons and divalent cations compete for a binding site within the channel pore, and the absence of these cations might allow protons to permeate the channel and to bind to specific intracellular inhibitory sites, the same bound by intracellular protons, leading to channel inhibition[85,88]. Interestingly TRPM7 is also sensitive to hypoxia as demonstrated by Tymianski group, demonstrating that TRPM7 is activated by anoxic conditions, as well as Mori's group, where TRPM7 is activated by following treatment with an hypoxic solution prepared by bubbling N_2 gas[89,90] (Table 1).

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Less data is available for TRPM6, however similarly to TRPM7 contrasting data are reported. TRPM6 is indeed inhibited by extracellular acidity similarly to TRPM7 in isolated pig myocytes[84] but potentiated by extracellular acidic pH in HEK293 cells, even though the magnitude of increase in TRPM7 inward current was higher than that of TRPM6[86] (Table 1).

Although evidence of effect of acidic pH on TRPM6/7 in cancer cells is lacking, several evidence has proved that TRPM7 is upregulated in several cancers and it is involved in the enhancement of a variety of cancer-related processes regulated by Ca^{2+} signaling, as proliferation, migration, invasion, cell death escape and survival and epithelial-mesenchymal transition (EMT), via the activation of the Ras-ERK and the PI3K/AKT/mTOR signaling pathways[80] (Fig. 1 and Fig. 3). Consequently, its activation by cancer-related extracellular acidic pH highlights its pivotal role in cancer progression. On the other hand, the ubiquitous expression of TRPM7 may also be taken in account for a specific targeted therapy.

2.2.2. TRP vanilloid subfamily

TRPV1 is a non-selective cation channel with relatively high permeability to Ca^{2+} ions, which is a major player in pain perception activated by different factors, including heat, inflammation and acidic environment as revealed by David Julius that shared the 2021 Nobel Prize for Physiology or Medicine[91–93]. Indeed, TRPV1 plays a key role in acidosis-induced pain, acting as a proton channel and being directly activated by protons[94,95].

TRPV1 is upregulated in cancer and regulates different cancer cell processes, such as proliferation, cell fate, migration and invasion, in a cancer type-specific manner and via the activation of different $[Ca^{2+}]i$-dependent signalling pathways, such as PI3K/AKT, Ras-ERK and JAK/STAT signalling cascades and NF-kb activation[96], acting as anti-proliferative and pro-apoptotic factor in melanoma, colorectal, pancreatic and liver cancer, among others, and exerting a pro-tumour role in highly aggressive types of cancer[96] (Fig. 1 and Fig 3).

Acidosis modulates TRPV1 activity, promoting its activation and potentiating its response to 2-APB, heat and capsaicin[95,97,98] (Table 1). In fact, hTRPV1 is activated by mild acidosis (pH_e 6.1), increasing intracellular Ca^{2+} levels, while the channels is blocked in presence of strong acidic conditions[98], identifying T633 residue in the pore helix and V538 residue in the S3–S4 linker as key residues involved in extracellular pH sensing[99]. Low pHe (< 5.9) significantly potentiate heat and capsaicin-evoked response in HEK293 cells, by increasing the channel's open probability at room temperature, therefore by lowering the threshold for the channel activation, even in absence of chemical stimuli[95]. This means that the potentiating effect of capsaicin and protons on TRPV1 are independent on each other, and they are mediated by different TRPV1 residues (Table 1 and Fig. 1). Concerning intracellular acidification, it enhances TRPV1 currents evoked by 2-APB, without affecting the ones induced by capsaicin, a selective TRPV1 agonist[97]. Extracellular acidic pH on the other hand activates TRPV1 and en-

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hances lymphatic endothelial cells' proliferative, migratory and invasive abilities via activation of NF-kB transcription factor and consequent upregulation of IL-8, a lymphangiogenic factor, contributing to lymphatic metastasis in tumour acidic microenvironment context[100] (Fig. 1). The pro- and anti-tumour effects of TRPV1 can be attributed to the different opening states available to the channel in response of different stimuli, exploiting each opening state's specific properties for the switching on of specific Ca^{2+}-dependent signaling pathways in different cancer cell types. Consequently, the pHe regulation of TRPV1 might be considered as cell type, ligand, and context specific, making it more difficult to identify its potential role as pharmacological target.

In addition to TRPV1, also **TRPV2**, **TRPV3** and **TRPV4** activity is modulated by acidic pH. **TRPV2** is an intracellular-resident non-selective cation channel that translocate to cell membrane following PI3K activation. Here, TRPV2-mediated Ca^{2+} entry regulates different physiological cellular processes. TRPV2 deregulation has been linked to several types of cancer, where its activity supports its progression, in particular via the activation of the PI3K/AKT and the ERK signaling cascades, by escaping cell death, increasing proliferation, cell migration and invasion[101]. TRPV2 is known to be insensitive to low pH_e alone[97,99], however, it has been demonstrated that acidic pH_e (6.0 and 5.5) potentiates TRPV2 currents in transiently transfected HEK293 cells that are evoked by 2-APB by modifying it, increasing the channel's sensitivity to this ligand from the cytoplasmic side, as proved by inside-out patch configuration[97] (Table 1 and Fig.1).

TRPV3 is a non-selective calcium permeant cation channel mostly expressed in brain and skin, where it is involved in chemo-somatosensing. TRPV3 oncogenic activity was demonstrated in lung cancer, where TRPV3 expression was associated with short overall survival and Ca^{2+}-mediated increased proliferation via Ca^{2+}/calmodulin-dependent kinase II (CaMKII)[102]. As for TRPV2, acidic pH_e alone (pH 5.5) is not able to activate the channel in HEK293 cells, but only to potentiate the TRPV3 response to 2-APB and its analogues via acidification of the intracellular milieu, increasing cytosolic Ca^{2+} levels [97](Table 1 and Fig. 1). Moreover, cytosolic protons activate the channel, inducing small but detectable currents, via a different mechanism respect to 2-APB response potentiation, and indicating four residues in the S2-S3 linker to be implicated in the acid intracellular activation of TRPV3[97]. A more recent study from the same group has elucidated the mechanism of TRPV3 acid intracellular activation and extracellular inhibition. The authors identified Asp641 residue, localized in the selectivity filter, as a critical residue involved in TRPV3 extracellular acidic pH_e inhibition. Intracellular acidification protonates E682, E689, and D727 residues in the C-terminal, facilitating channel's sensitization[103]. These data was also obtained by a previous work on TRPV3-transfected HEK293 and in HaCaT cells, where it was shown that TRPV3 is directly activated by glycolic acid-induced cytosolic acidification, inducing cell death, while extracellular acidification failed to activate the channel, resulting instead in decreased current amplitude[104] (Table 1 and Fig.1 and 3).

TRPV4 is a heat-activated and mechanosensitive channel that is deregulated in different cancer cells, acting mostly as a pro-tumour factor, enhancing cancer cells' migra-

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tion and metastasis through the activation of AKT and Rho/ROCK1/cofilin cascade, extracellular remodelling, proliferation and angiogenesis via activation of NFAT and PI3K signaling pathway[105–111], although a tumour-suppressive role has been also reported, especially in tumours expressing high TRPV4 levels[112,113] (Fig. 1 and Fig. 3).

For what concerns TRPV4 regulation by pH, low extracellular pH (pH_e 6) induces opening of transiently expressed TRPV4 in Chinese hamster ovary cells, with a maximal potentiation at pHe 4, as demonstrated by patch clamp current recording in absence of extracellular Ca^{2+} ions[114]. An opposite effect was observed in mouse oesophageal epithelial cells, where Ca^{2+} imaging experiments showed that the Ca^{2+} influx mediated by TRPV4 was abolished at pH_e 5[115] (Table 1). These opposite results might be explained considering the different techniques used by the two research groups and the experimental conditions in general. TRPV4 is a non-selective ion channel, permeable to protons when the extracellular solution is free from other divalent ions, such as Ca^{2+} ions. Since protons may compete with Ca^{2+} ions, a high extracellular proton concentration might lead to a decrease in Ca^{2+} influx but contributes to the gross TRPV4 current[115]. Collectively, considering the evidence outline above, TRPV4's role in tumour biology is cancer type specific and it might emerge as a potential drug target in the context of cancer treatment.

pH also regulates the activity of another component of TRP vanilloid family, **TRPV6**, a highly Ca^{2+} selective channel (PCa/PNa ~ 100) that is upregulated in different epithelial cancers, such as prostate, pancreatic, breast and ovarian cancer, in particular during early stages of tumour progression[116]. Several evidence has revealed the positive effect of TRPV6 activity on tumour progression through the activation of Ca^{2+}-dependent signalling pathways[116], promoting cancer proliferation and cell survival in prostate cancer cells by activating the Ca^{2+}-dependent NFAT transcription factor [117,118], invasion of breast cancer cells through Ca^{2+}/Calmodulin (CaM)-dependent kinases, such as CaMKII [119,120], cell survival, proliferation, and invasion in pancreatic cancer cells[121] and tumour growth in in vivo ovarian adenocarcinoma xenograft mouse model[122] (Fig. 1 and Fig. 3).

TRPV6 is a polymodal sensor, being regulated by different chemical and physical stimuli, including acidic pHe. Alkaline pH_e positively modulates TRPV6's activity, as demonstrated in Jurkat T-cells, where whole-cell patch-clamp experiments showed that solution at pH 8.2 determined the increase of TRPV6 activity and Ca^{2+} entry, while opposite effects were experienced for acidic pH_e (pH=6), which reduced inward TRPV6 currents and Ca^{2+} influx in Jurkat T-cells[123] (Table 1).

Inhibition of TRPV6 by extracellular acidification might be explained considering TRPV6 expression in distinct stages of cancer progression. Tumour can be characterized by a more acidic extracellular microenvironment during late stages[124], phases in which TRPV6 expression is downregulated in some types of cancers, such as colon cancer[125]. However, the lack of further studies focusing on the effect of extracellular acid pH on TRPV6 activity makes a comprehensive understanding of the role of acid pHe on TRPV6 in the context of tumour acidosis difficult.

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2.2.3. TRP ankyrin subfamily

TRPA1 is a non-selective cation channel that functions as a polymodal sensor, which deregulation is observed in several malignancies in a tissue-specific manner[126]. TRPA1 has been described as highly sensible to O_2 and oxidants in vagal and sensory neurons. In particular TRPA1 is activated in hypoxic condition by the relief of the inhibition of prolyl hydroxylase (PDH) which is O_2 sensitive[127].

The role of TRPA1 as ROS sensor has been demonstrated also in cancer cells by the same group: the work of Takahashi et al. has demostrated a key role of TRPA1 in promoting resistance to ROS-producing chemotherapies and oxidative stress tolerance in breast cancer cells via ROS-mediated TRPA1 activation and Ca^{2+}-CaM/PYK2 signaling pathway[128] (Table 1 and Fig 1). TRPA1 interaction with fibroblast growth factor receptor 2 (FGFR2) induces the activation of the receptor, promoting cancer cells' proliferation and invasion, prompting lung adenocarcinoma metastasization to the brain[129]. TRPA1 activation might also promote prostate cancer progression by triggering prostate cancer stromal cells secretion of VEGF[130], a known mitogenic factor involved in proliferation, migration and invasion of prostate cancer cells[131]. In line with those results, TRPA1 is indeed also an important player in promoting angiogenesis both in physiological retinal development as well as in prostate cancer derived endothelial cells[132] (Fig.3).

Besides being activated by oxidants, TRPA1 is activated by several distinct exogenous and endogenous compounds[133] and also by protons in the extracellular environment, which regulation is specie dependent. HEK293 cells expressing human and rodent TRPA1 showed a specie-specific activation of the channel, where only hTRPA1 generates a membrane current when exposed to different extracellular acidic environments (pH_e 6.4-5.4) reaching the maximal response at acidic pH_e 5.4; this effect specific for hTRPA1 was confirmed by Ca^{2+} imaging experiments in HEK293 cells as well as in DRG neurons derived from TRPV1/TRPA1-/- mice and overexpression hTRPA1 and neuroblastoma ND7/23 cells expressing hTRPA1, where only hTRPA1 induced an increase in Ca^{2+} entry when exposed to acidic pH_e. This specie-specific activation of TRPA1 is due to valine and serine residues within transmembrane domains 5 and 6[134] (Table 1).

TRPA1 can also be activated by intracellular acidification, as observed in the context of ischemia-induced acidification of the extracellular microenvironment in mice oligodendrocytes, with following acidification of the intracellular space and activation of TRPA1. This leads to an increase of Ca^{2+} influx and damage to myelin[135] (Table 1). Altogether, these results and its role in cancer suggest that TRPA1 activation by acidic pH^e may play a significant role in promoting cancer progression, highlighting its potential as a therapeutic target.

2.2.4. TRP canonical subfamily

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TRPC1 is a non-selective cation channel, which assembles to form homo- and heteromeric channels with other members of the family, such as TRPC3, TRPC4 and TRPC5. TRPC1 up-regulation has been reported in several cancers, such as pancreatic cancer, where it potentiates BxPc3 cells' migration via Ca^{2+}-dependent activation of PKCα[136], breast cancer, where it exerts a pro-proliferative role in MCF-7 cells by mediating Ca^{2+} influx induced by $K_{Ca}3.1$ activation[137] and via Ca^{2+}-dependent ERK1/2 activation[138]. TRPC1 also promotes human glioma cancer cells' proliferation via Ca^{2+} entry and supports tumour growth in vivo[139], and lung cancer differentiation, by promoting A549 cell proliferation[140]. TRPC1 also participates with ORAI1 channels in the induction of vimentin, a mesenchymal marker for epithelial-mesenchymal transition (EMT)[141] (Fig. 2 and Fig. 3).

TRPC1 is not regulated by protons but by hypoxia, mediating its response in the regulation of gene expression. The study of Wang B. et al. of 2009 have reported that TRPC1 is functionally expressed in U-87 malignant glioma cells under hypoxia, where it promotes the up-regulation of VEGF expression, as VEGF mRNA levels were significantly decreased in presence of TRPC1 inhibitor or RNAi in hypoxic conditions[142] (Table 1 and Fig 2). VEGF has a central role in angiogenesis in both physiological and pathological conditions[143] and solid tumours are characterized by a hypoxic microenvironment, in which the lack of oxygen might promote VEGF expression, in order to induce angiogenesis and to increase tumour oxygen supply. TRC1 mediates hypoxia responses also in breast cancer cells, where HIF-1α promotes its up-regulation. In MDA-MB-468 breast cancer cells, TRPC1 is involved in the transactivation of Epidermal Growth Factor receptor (EGFR) during hypoxia, leading to the increase of LC3B autophagy marker[144]. Moreover, hypoxia-induced TRPC1 activation promotes epithelial-mesenchymal transition in the same cells, up-regulating the mesenchymal marker Snail and down-regulating the epithelial marker claudin-4, promoting the hypoxia-induced EMT and, therefore, the aggressive and invasive phenotype of breast cancer cells[144] (Table 1 and Fig 2).

TRPC5 is a Ca^{2+}-activated ion channel which is regulated by components of the tumour microenvironment, such as acidosis, and supports hypoxia responses. In long-term adriamycin-treated breast cancer cells, TRPC5-mediated Ca^{2+} influx promotes HIF-1α translocation in the nucleus and therefore the downstream transcription of HIF- 1α-regulated VEGF expression, highlighting its contribution in promoting breast cancer angiogenesis[145] (Table 1 and Fig 2). The same research group also validated TRPC5 role in mediating HIF-1α response in tumour progression in colon cancer, where TRPC5 activates the HIF-1α-Twist signaling pathway to promote EMT, migration and proliferation in SW620 colon cancer cells[146]. TRPC5 also acts as a pHe sensor, as its spontaneous activity and G protein-activated currents are potentiated by extracellular acidic pH by increasing the channel open probability in presence of small changes of extracellular pH, with a maximum activity around pHe 6.5 and current inhibition starting from pH 5.5[147] (Table 1).

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Potentiation by acidic pH_e and involvement in HIF-1α regulation demonstrate the interest of cancer cells in keeping TRPC5 channels active and over-expressed, in order to promote cancer specific hallmarks, in particular chemoresistance. Indeed, the role of TRPC5 in promoting chemoresistance in different types of cancer is well known. In breast carcinoma cells, adriamycin -induced TRPC5 upregulation protects them from chemotherapy treatment, by inducing autophagy via increase in cytosolic Ca^{2+} concentrations and activation of Ca^{2+}-dependent CaMKKβ/AMPKα/mTOR pathway, promoting the cancer cell survival and tumour growth in vivo[148] (Fig. 2). The role of TRPC5 in therapy resistance is not confined to breast cancer, as its upregulation has been also identified in 5-fluorouracil resistant human colorectal cancer cells, where TRPC5 overexpression determines the overproduction of ATP-binding cassette subfamily B member 1 (ABCB1), a pump involved in drug resistance through the export of cytotoxic drugs, via Ca^{2+} entry and activation of Ca^{2+}-dependent Wnt/β-catenin signaling pathway and in a glycolysis-dependent manner[149,150] (Fig. 2).

The work of Ma X. et al. of 2012 has also demostrated, *in vitro* and *in vivo*, the critical role of this channel in promoting chemoresistance of adriamycin-resistant MCF-7 breast cancer cell line with the up-regulation of another pump linked to drug resistance. TRPC5 overexpression results indeed in Ca^{2+} influx and activation of P-glycoprotein overproduction, a pump in charge of removing cytotoxic drugs from cells via Ca^{2+}/calmodulin/calcineurin-dependent NFATc3 signaling pathway[151]. Beside chemoresistance, TRPC5 expression was observed to be positively correlated with high proliferative, migratory, and invasive abilities of colon cancer cells, promoting the EMT through the HIF-1α-Twist signaling pathway[146]. Therefore, these results demonstrate that the impact of the acid and hypoxic cancer microenvironment on TRPC5 channel is aimed at its activation, thus promoting tumour progression (Fig. 3).

Similar behaviour is shown by **TRPC4**, which shares a high sequence similarity with TRPC5. TRPC4 is a Ca^{2+}- and G-coupled receptors-activated non-selective Ca^{2+} permeable cation channel [152]. TRPC4's role in cancer has been elucidated in the last years, being involved in promoting angiogenesis via cytosolic Na^+ and Ca^{2+} rise[153,154] and proliferation[155], although a negative effect on A-498 renal cell carcinoma cells via Englerin A-mediated channel activation has been documented[156].

TRPC4-mediated currents are 2-fold potentiated when exposed to pHe 6.5, however more acidic solutions lead to current inhibition starting from pHe 6, with complete current inhibition at pH_e 5.5[147]. $G_{i/o}$-mediated TRPC4 activation is also accelerated by intracellular protons in an indirect way, regulating the kinetics of $G_{i/o}$-dependent TRPC4 activation, and it requires an increase in intracellular Ca^{2+} concentration. Intracellular protons do not act directly on the channel, as they inhibit TRPC4 activation by its direct agonist, Englerin A, but by acting on PLCδ1[157] (Table 1; Fig. 2 and 3).

TRPC6 is another TRPC member to be regulated by tumour microenvironmental clues, such as hypoxia. In fact, hypoxic conditions enhance TRPC6 expression in murine pancreatic stellate cells, which constitute the major cellular components in pancreatic ductal adenocarcinoma's stroma, and which play a key role in generating PDAC's characteristic desmoplasia. TRPC6 promotes their activation and it is involved in the

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secretion of pro-migratory factors in presence of hypoxia[158]. Hypoxia up-regulates TRPC6 mRNA expression in hepatocellular carcinoma cells, where TRPC6-mediated Ca^{2+} influx confers drug resistance to these cells via Ca^{2+}-dependent STAT3 signaling pathway in hypoxic conditions[159]. Hypoxia activates Notch1 and downstream TRPC6 expression also in glioma cells, with consequent rise in cytosolic Ca^{2+} concentration and Ca^{2+}-dependent activation of calcineurin-NFAT signaling pathway, promoting proliferation, cell invasion and angiogenesis under hypoxia[160] (Table 1; Fig. 1 and 3). Hypoxia also upregulates TRPC6 in hepatic stellate cells via HIF-1α/Notch1 pathway, leading to TRPC6-mediated Ca^{2+} influx and the downstream activation of Ca^{2+}-dependent nuclear factor of activated T-cells (NFAT) transcription factor and SMAD2/3- dependent TGF-β signaling, which activation results in the expression of ECM proteins, such as collagen type I, that facilitate hepatic stellate cells' fibrotic activation and promotes hepatic fibrosis, strongly linked to arise of hepatocellular carcinoma[161] (Table 1; Fig. 1 and 3).

As regarding the direct role of acidic extracellular pH (around pH 6.5), it is sufficient to inhibit TRPC6, and the inhibition increases in a pH-dependent manner, affecting both inward and outwards currents in HEK-transfected cells[147] (Table 1). This inhibitory effect of acidic pH_e might be explained considering the high TRPC6 levels expressed by pro-tumorigenic immune cells, such as neutrophils, where TRPC6-mediated calcium entry is required for CXCR2-mediated intermediary chemotaxis[162]. Consequently, inhibition of TRPC6 by acidic pH_e may impair neutrophils' migration and prevent them from leaving the acidic tumour microenvironment, thus contributing to its progression and metastasis by releasing ROS, secreting pro-tumour factors and inducing drug resistance[163]. Altogether, these results suggest that the acidic pH_e-mediated potentiation of TRPC channels might be restricted to only some members, and it depends on the cell type expressing the channels.

2.2.5. Store-Operated Ca^{2+} Channels

ORAIs are Ca^{2+}-release activated Ca^{2+} channels which are major players, with **STIM** proteins, in the mechanism known as store-operated Ca^{2+} entry (SOCE), which mediates Ca^{2+} entry into cells promoting the refilling of ER calcium stores as well as intracellular signaling, controlling both physiological and pathological processes such as cell motility, cell proliferation, gene expression, apoptosis escape and cell invasion[164–166]. SOCE represents the main route of Ca^{2+} entry in different types of cancer cells, contributing to several cancer hallmarks. Indeed, different works have highlighted the key role of SOCE in promoting migration of different cancer cell lines, such as chemoresistant IGROV1 ovarian cancer cells by regulating focal adhesion turnover[167], SW480 colorectal carcinoma cells[168] and of oral cancer cells through Akt/mTOR/NF-κB signalling[169]. Moreover, SOCE has been implicated in enhancing invasion of triple negative breast cancer cells, as well as angiogenesis and migration, through NFAT4 signalling[170] and through NFATc3 in colorectal cancer cells and tissues from patients[171], while in WM793 cells melanoma cells, SOCE-induced Ca^{2+} oscillations con-

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tribute to invadopodia formation via Src activation[172]. SOCE might promote invasion of cancer cells by inducing epithelial-mesenchymal transition, as observed in DU145 and PC3 prostate cancer cells[173] and BGC-803 and MKN-45 gastric cancer cells[174]. Store Operated Channels (SOCs) in general play a key role also in the modulation of sensitivity to chemotherapy in a cancer type-specific manner, by promoting chemo-resistance in breast cancer cells in the case of ORAI3[175], ORAI1 and STIM1 in pancreatic ductal adenocarcinoma[165], ORAI1 in hepatocarcinoma[176], among others. SOCE also promotes extracellular vesicles formation, which are signalling vectors involved in the intercellular acquisition of multidrug resistance, in both malignant and non-malignant breast cancer cells via activation of calpain[177].

Beside all these contributions to cancer progression, SOCE mediates hypoxia and pH-induced functional changes. Several studies have demonstrated that intracellular and extracellular pH are able to modulate the activity of ORAI channels by affecting its coupling with STIM1 and/or by modifying its gating biophysical properties. Results obtained by numerous studies conducted on ORAI/STIM have clarified the concept that both intra- and extracellular acidic pH has an inhibitory effect on the activity of ORAIs, and on SOCE in general, while intra- and extracellular basic pH potentiate them. The notion that extracellular pH regulates native CRAC currents (I_{CRAC})was already known in 1995, where the work of Malayev A. and Nelson D.J. has shown that acidic extracellular pH (pH_e= 6) decreases the amplitude of inward Ca^{2+} currents while basic pH_e (pHe= 8) increases it in macrophages by using Patch-clamp technique, and that these changes were reversible and voltage-independent[178] (Table 1). More recently, inhibition by acidic pH_e was demonstrated in H4IIE rat liver cells overexpressing ORAI1 and STIM1 proteins, in which I_{CRAC} were inhibited completely at pH_e 5.5[179] (Table 1). In the same work, researchers identified Glu106, located in ORAI1's pore, as the residue responsible for pH dependence of CRAC currents, as E106D mutation in ORAI1 abolished the inhibition of I_{CRAC} by acidic pH_e. These results were also supported by work of Beck A. and colleagues, which demonstrated with whole-cell patch clamping that extracellular as well as intracellular acidification decreases amplitude of IP3-induced endogenous I_{CRAC} in RBL2H3 mast cell line and Jurkat T lymphocytes and in heterologous ORAI/STIM-mediated I_{CRAC} in HEK293 cells[180] (Table 1). In contrast to acidification, external alkalinisation increases both endogenous and overexpressed ORAI/STIM amplitude of I_{CRAC} (pKa of 7.8 for RBL2H3 mast cells, 8.0 for Jurkat T lymphocytes and 7.9 for HEK293 cells). In this work, researchers identified other key residues located in ORAI1's first extracellular loop that contribute to some extent to pH_e sensitivity; D110 and D112, as mutations of these residues to alanine prevent the alkalinisation-induced potentiation of I_{CRAC} and increases its amplitude in presence of acidic pH_e[180]. Enhancement and decrease of SOCs activity by external basic and acidic pH, respectively, were further confirmed in heterologous ORAI/STIM-mediated currents in HEK293 cells by Tsujikawa H. et al. in 2015, which have also demonstrated that E106 mediates pH_e sensitivity when Ca^{2+} is the permeant cation, while E190 when Na+ is the permeant cation[181] (Table 1).

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The effect of alkaline pHi on I_{CRAC} is controversial. Indeed, while intracellular alkalization did not significantly increase both IP3-induced endogenous and heterologous ORAI/STIM-mediated I_{CRAC} amplitude[180], alkaline pH_i-mediated potentiation of ORAI1/STIM1 activity was observed in other papers[181,182] (Table 1). These differences might be explained considering the type of intracellular Ca^{2+} buffer used[182]. Moreover, cytosolic alkalinisation leads to SERCA inhibition, resulting in Ca^{2+} release from ER stores and activation of SOCs, with Ca^{2+} influx in NIH 3T3 cells[183]. Residue H155 located in the intracellular loop of ORAI1 is responsible for ORAI1/STIM1 pH_i sensitivity, as mutation to phenylalanine decreases low pH_i-mediated I_{CRAC} inhibition and alkaline pH_i-mediated I_{CRAC} potentiation[181]. Since the effect of pH was the same in presence of all ORAI isoforms[180] and that both extracellular pH sensors (residues E106 and E190) and intracellular one (residue H155) are conserved in all three ORAI isoforms, it might suggest that the residues mentioned before act as common pH_i and pH_e sensors in ORAI1-2-3/STIM isoforms. Beside H155, negatively charged amino acid residues in STIM1 inactivation domain play an important role in pH_i sensitivity[182].

For what concerns hypoxia-mediated regulation of SOCs, intracellular acidification of Primary Aortic Smooth Muscle Cells and HEK293 cells induced by hypoxia leads to inhibition of SOCE by disrupting the electrostatic ORAI1/STIM1 binding and closing ORAI1 channel (Table 1 and Fig 2). Nonetheless, STIM1 remains associated with ORAI1 through the second binding site located between ORAI1's intracellular N-terminal tail and STIM1's STIM-ORAI activating region (SOAR), preventing the noxious hypoxia-mediated Ca^{2+} overload[184]. Therefore, intracellular acidification might regulate SOCs activity by uncoupling ORAI11 and STIM1 and, consequently, reducing I_{CRAC} amplitude.

As ORAI/STIM mediates most of Ca^{2+} signaling in cancer-induced acidosis, they play a key role in several pathological processes. In cancer context, it was observed that SOCE is regulated by changes in extracellular pH, as acidification of tumour microenvironment suppressed both the carbachol- and thapsigargin-mediated Ca^{2+} entry in neuroblastoma cells, while external alkalinisation increased both the CCH- and the TG-induced Ca^{2+}-influx[185], therefore in accordance with the results obtained in non-cancer cells (Fig. 2).

However, the work of Liu X. et al. of 2018 on triple negative breast cancer cells (TNBCs) reported that hypoxia promotes the activation of Notch1 signaling, required for the up regulation of ORAI1 mRNA and protein levels in TNBCs (Fig. 2). In addition, up-regulation of ORAI1 in hypoxia determined an increase of basal Ca^{2+} concentration and of thapsigargin-induced SOCE, therefore of intracellular Ca^{2+} concentration in TNBCs, which activated the downstream NFAT4 target, known to regulate the expression of cancer-related genes involved in its hallmarks[170]. Results of the same work showed that hypoxia enhanced invasion and TNBCs migration via Notch1/ORAI1/SOCE/NFAT4 pathway, therefore, ORAI1 and SOCE play a key role in promoting an aggressive phenotype. Same results were obtained in colon cancer cells by the same group of researchers, where potentiation of SOCE mediated by hypoxia-induced upregulation of ORAI1 determined the activation of NFATc3, enhancing hy-

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poxia-induced invasion and angiogenesis in colon cancer cells[171]. The hypoxia-induced upregulation of SOCE components was also demonstrated in A549 and NCI-H292 non-small cell lung cancer cells, where nicotine treatment determined the upregulation of HIF-1α, which increases the expression of the SOCE components TRPC1, TRPC6 and ORAI1. This translates in potentiation of SOCE and calcium entry, promoting lung cancer cells' proliferation[186].

Considering all the previous results shown, it is evident that they are quite contradictory. Several studies have shown that ORAI/STIM and SOCE promote cancer development[187] and that also tumour acidosis and hypoxia support it, by enhancing tumour cell migration, invasion and, therefore, its aggressive phenotype[40,188]. Hence, the inhibitory action of the tumour intra- and extracellular acidic pH on SOC channels seems to contradict the positive effect of SOCE on tumour progression. Possible explanations about the apparent counterproductive blockade of SOCE channels by the tumour acidic pH lie in the fact that Ca^{2+} signaling not only promotes tumour progression and development by potentiation its hallmarks, but it also contributes to its suppression by enhancing processes as cell death, senescence and autophagy[189]. In addition, ORAI members assembly to form different combinations of heteromeric Ca^{2+} Release Activated channels (CRACs) and the ratio of each ORAI member determine specific I_{CRAC} current properties and CRAC effects[190,191]. Therefore, the acidic pH of tumour microenvironment may differently regulate heteromeric CRACs. Another point to consider is the key role of SOCE in immune cell activation[192]. The requirement of Ca^{2+} entry for antitumour immunity might explain the inhibitory effect of acidic tumour microenvironment on SOCE, in order to decrease immune cells' function and protect the tumour.

3. Concluding Remarks

The present review aimed to overview the link between major chemical components of the tumour microenvironment, hypoxia and acidic pH_e, and Ca^{2+}-permeable ion channels, summarizing the major Ca^{2+}-mediated signaling pathways that are involved in hypoxia and acidic pH_e responses. We focused our attention mainly on no-voltage gated TRP, SOCs as well as Piezo channels due to their role as polymodal sensors in tumour microenvironment. In this perspective we have seen that hypoxia has a positive effect on some TRP and ORAI1 channels (Fig. 3), promoting their activation and their expression via different transcription factors. At the contrary, tumour acidosis modulation shows a higher variability, determining loss-of-function of specific Ca^{2+}-permeable ion channels expressed not only by cancer cells but also immune cells, or potentiation or activation of others. Although the roles of tumour acidosis and Ca^{2+} signalling in cancer progression are well established, little research has been performed to address their link in the context of tumorigenesis, with the majority of works cited in this review performed on tumorigenic cell lines and with a focus on acidic pH_e effect on Ca^{2+}-permeable ion channels' current via electrophysiology experiments. In addition, little information about intracellular acidification regulation of Ca^{2+} signals and the pH_i-

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dependence of Ca^{2+}-permeable channels in cancer is available, although it is well known that it is the intracellular pH that plays a more decisive role in regulating various biological processes, and that this value is highly influenced by the extracellular pH. Therefore, the putative synergistic relationship between hypoxia, tumour acidosis and low pH_e-induced intracellular acidification and Ca^{2+} signals, the mechanisms of their interaction and their interdependence in tumours require further studies and clarifications, in order to fill the gap and promote a better understanding of the crosstalk between three major players in the cancer research field.

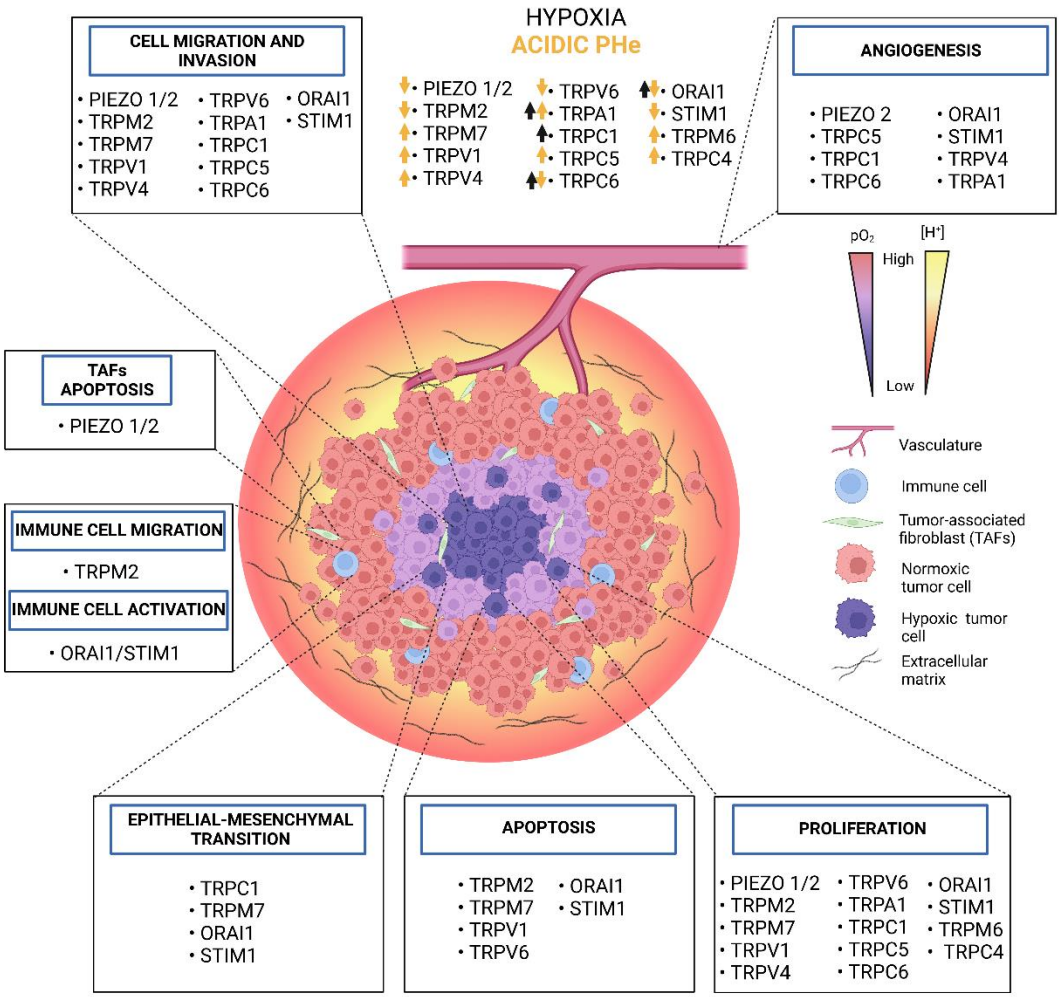

Figure 3. Schematic illustration of tumour microenvironment landscape, characterised by a solid tumour that undergo a series of genetic rearrangements, that modify cancer cells' phenotype towards more aggressive ones, resulting in an increased lactate production and secretion that acidify the extracellular milieu, in concomitance with other processes that determine H^+ production. The increased tumour growth, the acidification of the extracellular space and the aberrant vascularization and limited O_2 supply origin a tumour core that is hypoxic and acidic, with limited supply of oxygen and nutrients from the blood vessels. Peripheral tumour cells are located in regions with a higher extracellular pH, a result of proximity to blood vessels and the possibility to

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wash out acidic waste products. Cancer, immune and stromal cells express Ca^{2+}-permeable ion channels which act as microenvironmental transducers, sensing TME cues and being inhibited or activated/potentiated by hypoxia and/or acidic pH_e, leading to the activation or suppression of Ca^{2+}-dependent downstream signalling pathways involved in different cancer hallmarks, such as proliferation, migration, invasion, angiogenesis, and epithelial-mesenchymal transition.

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