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Mechano- and pH-sensing convergence on Ca^{2+}-mobilising proteins – A recipe for cancer?

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ABSTRACT

Alterations in the (bio)chemical and physical microenvironment of cells accompany and often promote disease formation and progression. This is particularly well established for solid cancers, which are typically stiffer than the healthy tissue in which they arise, and often display profound acidification of their interstitial fluid. Cell surface receptors can sense changes in the mechanical and (bio)chemical properties of the surrounding extracellular matrix and fluid, and signalling through these receptors is thought to play a key role in disease development and advancement. This review will look at ion channels and G protein coupled receptors that are activated by mechanical cues and extracellular acidosis, and stimulation of which results in increases in intracellular Ca^{2+} concentrations. Cellular Ca^{2+} levels are dysregulated in cancer as well as cancer-associated cells, and mechano- and proton-sensing proteins likely contribute to these aberrant intracellular Ca^{2+} signals, making them attractive targets for therapeutic intervention.

1. Introduction

The (bio)chemical and physical environment are crucial determinants of cell and hence tissue fate. Appropriate and coordinated changes in the (bio)chemical composition and mechanical properties of the extracellular space are an absolute requirement for and accompany normal cell and tissue development [1–3]. Unsurprisingly, therefore, aberrant changes in them contribute to disease formation and progression. One disease for which impact of mechanical and biochemical dysregulation is particularly well established is cancer. Solid cancer development and progression is accompanied by changes in the mechanical properties of the tumour tissue and acidification of its interstitial fluid [2,4–7], and transformed cells as well as cancer-supporting cells (e.g. cells making up tumour vasculature, infiltrating immune cells, cancer-associated fibroblasts, CAFs) are characterised by dysregulated intracellular Ca^{2+} signals [8–10]. This review will look at Ca^{2+}-permeable plasma membrane ion channels and G_q-coupled cell surface receptors that are activated by mechanical stress and extracellular acidosis. Activity of these receptors results in increases in intracellular Ca^{2+} concentration, and they are therefore likely to take part in creating aberrant intracellular Ca^{2+} signals that ultimately contribute to cancer formation and progression.

2. Mechanical cues and cancer

The most apparent difference between normal and cancerous tissue in terms of physical properties is the altered tissue stiffness of cancerous compared with healthy tissue; cancers can be over ten times stiffer than their host tissue [4]. This has two important consequences. First, increased stiffness enables tumours to displace surrounding tissue, allowing tumours to grow in size; the surrounding normal tissue is less able to resist deformation in response to pressure applied by the growing cancer than the cancer tissue is to resist deformation due to pressure caused by the surrounding healthy tissue [4,11,12]. Second, increased and increasing cancer stiffness imposes mechanical stress on the tumour itself and at the interface between tumour and healthy tissue. Tissue stiffness generates so-called solid stress (solid because it is generated by the non-fluid components of the tissue such as extracellular matrix (ECM) materials), defined as force per unit area. This force can either lead to compaction (compression) or expansion (tension) of the cells and hence tissue on which it acts [12]. For solid tumours, the increased tissue stiffness causes compression of the tumour interior whilst the interface between tumour and normal tissue experiences tension [11,12]. Several consequences arise from this. Compression of core cancer tissue compresses blood and lymph vessels and may even result in collapsed vessels [4,13]. In addition to somewhat erratic and abnormal blood vessel formation [14], this results in increased hypoxia and acidification of the affected part of the tumour,

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which can promote cancer cell survival and therefor tumour progression including metastasis [4,5,15,16]. Additionally, the inconsistent and irregular vessel architecture, low blood flow rates in tumour blood vessels, and higher ECM density, which increases diffusion distance and may act as a buffer for drugs, interfere with efficient drug delivery, thus promoting cancer progression by hindering treatment [17].

Conversely, cells and vessels at the tumour periphery experience tension rather than compression, and particularly lymph vessels are significantly enlarged, resulting in increased lymph flow. This potentiates the metastatic potential of the tumour as it promotes efflux of lymph and cancer cells traveling within the lymph away from the tumour tissue [18,19]. Finally, interstitial fluid pressure and hence fluid shear are increased in the core, and this, too, is thought to promote the metastatic potential of cancers [20]. Importantly, compression can trigger cell death [21], and this may contribute to cell death in the tumour core, which is generally considered to be a consequence of poor perfusion and lack of nutrients [22].

So why are cancerous tissues stiffer than their non-transformed counterparts? Increased tissue stiffness is the result of at least four distinct processes. First, aberrant ECM production and remodelling lead to increased tissue stiffness and can take the form of desmoplasia, which is generally thought to enhance cancer progression and can indicate worse clinical outcome for certain cancers [11]. Crucially, this altered deposition of fibrotic material may also limit access of T cells, thus interfering with the immune response and contributing to cancer development [23,24], though this may not be a general mechanism [25]. Second, cells within the tumour alter their intracellular Na^+ load. As a consequence of this osmotic adjustment, cancer cells swell, and this protects them from the negative impact of compression [26], as well as contribute to increased tissue stiffness. Third, hyaluronan, a negatively charged polysaccharide enriched in tumour ECM, attracts water molecules to create hydrated gel-like regions within the tumour that are capable of resisting the compressive stresses applied to core cancer cells [11,12]. Fourth, cells that are exposed to a stiff environment respond by adapting their own intracellular tension to balance the extracellular forces they experience, leading to increased cell and hence overall tumour stiffness [11,27]. Importantly, increased cell stiffness also permits cells to generate higher traction and thus enables cancer cells to migrate more effectively through tissues, thereby promoting metastasis [27–29]. A summary of these principles is provided in Fig. 1.

Mechanical forces not only affect cells within the cancerous tissue but also play a key role in the metastatic process. Cancer cells disseminating through the body by traveling in lymph or blood are exposed to fluid shear forces that are profoundly different from each other and from those experienced by cells in tissues. In particular, shear forces in blood can vary dramatically depending on the vessel type, architecture and location, and cancer cells hence have to adapt to withstand a range of distinct shear stress levels [30]. Furthermore, the target tissue is likely to have distinct mechanical properties compared with the primary cancer, posing yet another mechanical challenge or stimulus [30]. Additionally, epithelial-mesenchymal transition, a key event responsible for tumour progression and metastatic dissemination, is regulated by mechanical forces [31]. Since only a small population of cancer cells, the so-called cancer stem cells, are able to proliferate and generate tumour mass, only these cells can therefore be responsible for metastasis formation [32]. It hence seems reasonable to suggest that successfully metastasised cancer stem cells must be able to withstand a range of mechanical stresses, e.g. through adaptation of the cell's own mechanical properties (for instance to resist shear stress) and/or by eliciting cellular responses activated in response to mechanical stresses that promote cell survival and proliferation rather than cell death.

3. Extracellular acidosis and cancer

The interstitial fluid of solid tumours is significantly more acidic than that of the host tissue, and this is mainly due to the altered metabolic activity of transformed cells. Instead of relying on oxidative phosphorylation to generate ATP, cancer cells employ lactic acid-producing anaerobic glycolysis to generate ATP even under normoxic conditions [33]. Since ATP yield through glycolysis is lower, glucose consumption and hence lactic acid production is high in tumours. To keep intracellular pH (pH_i) neutral, which is essential for cell proliferation [6], cancer cells extrude lactic acid, resulting in acidification of the interstitial fluid. In fact, cancer cells keep their pH_i more alkaline than non-transformed cells (pH_i of 7.4 instead nearer pH_i7.2 in normal cells) through a variety of mechanisms, including H^+ extrusion via proton pumps and voltage-gated proton channels and import of bicarbonate from the interstitial fluid [6,33,34].

In addition, dysfunctional blood vessels cause erratic and insufficient O_2 supply to solid cancers, resulting in the creation of hypoxic regions that contribute to tumour interstitial fluid acidification via carbonic anhydrase (CA) IX activity [35]. These enzymes have an extracellular active site and hydrate extracellular CO_2 to generate protons and bicarbonate. The latter is then taken up by cells to neutralise intracellular acid via intracellular CA activity [36], leaving behind protons that enhance interstitial acidification [37]. CA IX is expressed following activation of the hypoxia inducible factor (HIF) pathway, which in turn is stimulated independently by hypoxic and acidotic conditions [38,39], resulting in a positive feedback cycle (Fig. 2).

As a consequence of all of these tumour-specific alterations, tumour interstitial fluid pH can reach values as low as pH_o5.8 depending on the tissue of origin [40], and for a given tumour type, there is a positive correlation between pH_o acidity and tumour size [41]. Furthermore, hyperglycaemic conditions can decrease tumour pH_o by up to one more

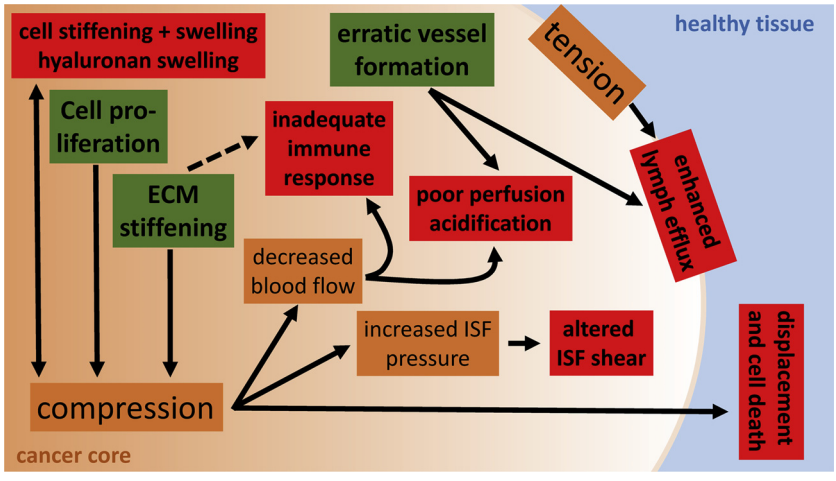

Fig. 1. Mechanical forces and their impact on solid tumours. Schematic summarises how different mechanical pressures (orange) and cancer-specific changes in cell behaviours (proliferation, erratic vessel formation, abnormal extracellular matrix (ECM) stiffness; green) come together to promote cancer progression and metastasis (red). See text for details. Dotted arrow indicates mechanism that may not be present in all solid tumours.

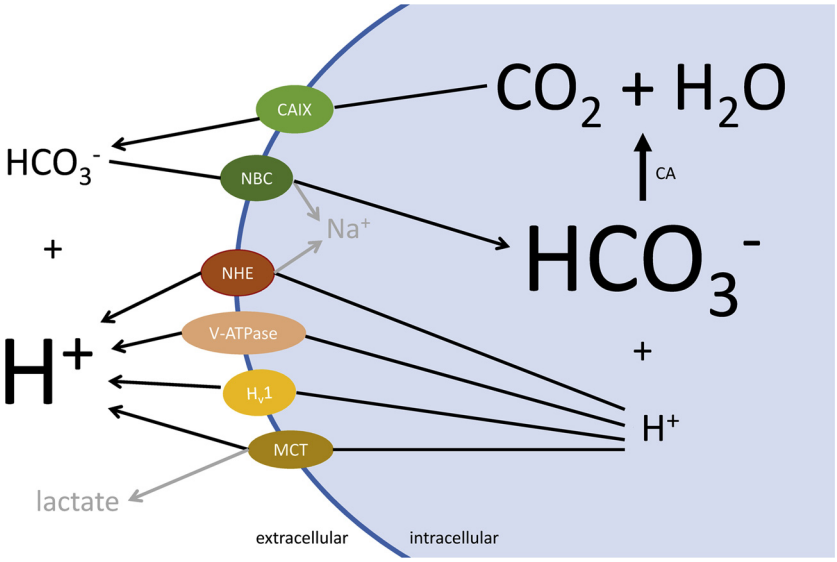

Fig. 2. Cancer cells acidify cancer interstitial fluid.
Cancer cells utilise a range of distinct transmembrane proteins to remove intracellular protons (H^+) and import extracellular bicarbonate (HCO_3^-). Intracellular carbonic anhydrase utilises bicarbonate to remove intracellular protons by catalysing their conversion to carbon dioxide (CO_2) and water. Carbon dioxide diffuses out of cells and, together with water, is converted back into extracellular bicarbonate and protons via transmembrane carbonic anhydrase IX. Together, this results in acidification of the extracellular fluid whilst the intracellular pH becomes more alkaline. Grey arrows and text indicate other ions or metabolites being carried by transporter/exchanger with protons.
Abbreviations: CA – carbonic anhydrase; NBC – sodium bicarbonate transporter; NHE – sodium proton exchanger; H_v1 – voltage-gated proton channel; MCT – lactate transporter.

pH unit [41], testament to the fact that glycolysis is a key contributor to tumour interstitial fluid acidification.

Importantly, cancer progression and metastasis do not occur despite but because of the acidic microenvironment [5,6]. Exactly how extracellular acidosis promotes cancer progression is still not fully understood. It is well established that interstitial acidity can increase resistance to drug treatment by promoting drug efflux through transporters and/or decreasing drug efficiency through protonation [42,43]. Furthermore, extracellular acidosis can stimulate autophagy as well as suppress infiltrating immune cell function, both of which facilitate cancer progression [6]. Moreover, exposure to acidotic conditions enables certain cancer cells to acquire enhanced motility and hence invasion potential that remains even when cells are exposed to physiological pH_o again [44]. However, it should be noted that in other cancer cells extracellular acidosis can inhibit these processes [45].

4. Intracellular Ca^{2+} signals and cancer

There is a plethora of evidence linking changes in intracellular Ca^{2+} signalling to cancer development and progression, and a role for aberrant Ca^{2+} signalling in cancer is well established and has been reviewed extensively in many different contexts. Briefly, expression and/or activity of many Ca^{2+} handling proteins (ion channels, pumps, buffers) is altered in cancer and contributes to cancer progression [46–51]. Furthermore, mutations in Ca^{2+} handling proteins are well documented (e.g. [52,53])) and likely contribute to transformation and cancer progression [54]. Consequently, abnormal intracellular Ca^{2+} signals and levels are observed in transformed and cancer-supporting cells, and this is linked to aberrant cell behaviours that enable cancers to grow and progression (e.g. [55–57]).

5. Transmembrane proteins involved in detection of mechanical cues and extracellular acidosis

Cells devote a large number and range of distinct transmembrane and intracellular proteins to the detection of mechanical cues and changes in extracellular proton concentration ($[H^+]_o$), i.e. extracellular pH (pH_o). Notably, detection of mechanical force and $[H^+]_o$ can converge at the level of a single protein. This review will focus on Ca^{2+}-permeable ion channels and G_q-coupled cell surface receptors whose function is promoted by extracellular acidification and mechanical force, and activity of which results in increases in intracellular Ca^{2+} concentration. Proton- and mechanosensitive proteins linking to intracellular Ca^{2+} signals are perfectly positioned to translate maladaptive extracellular changes into intracellular Ca^{2+} changes in the cells in which they are expressed and are hence likely to contribute to aberrant Ca^{2+} signals in cancer cells. Only those proteins that are the primary mediators of proton and mechanical stress-mediated changes in intracellular Ca^{2+} concentration are considered. It would be beyond the scope of this review to include all ion channels and receptors that are modulated in their activity by changes in extracellular pH and mechanical stress, or to include ion channels that may be activated or modulated in their activity downstream of the primary proton- and mechanosensitive receptor or ion channel.

5.1. Acid sensing ion channels (ASICs)

ASICs are proton-gated, non-selective cation channels that belong to the amiloride-sensitive epithelial Na^+ channel/Degenerin (NaC/DEG) superfamily of ion channels [58]. Five genes code for seven ASIC subunits (ASIC1a and b, ASIC2a and b, ASIC3, ASIC4, ASIC4) that can form homo- and heterotrimers, though ASIC4 and 5 homomers do not form functional channels. ASIC-medicated currents exhibit transient or biphasic responses to extracellular acidosis (ranging from pH_o7.0 to 6.0), meaning that they can signal both sustained and acute changes in pH_o [59]. Intriguingly, ASIC2 and 3 but not 1 have been implicated in mechanosensing in mice deficient for the respective gene [60]. How ASICs sense mechanical stress is not entirely clear but they appear to require the presence of stomatin-domain proteins, including stomatin and stomatin-like (STOML) 1 and 3, in order to function as mechanosensors [60]. Crucially, interaction of ASICs with stomatin-domain proteins interferes with their ability to respond to extracellular acidosis with inward currents [60]. This is a critical finding as it suggests that ASIC2 and 3 can only serve as either proton- or mechanosensors, but not both, and that therefore the presence of auxiliary proteins determines which of the two stimuli can promote activity of these channels.

Cultures of primary human high- but not low grade glioma cells or normal human astrocytes exhibited amiloride-sensitive, constitutive inward currents at physiological pH_o [61,62], that were ASIC1 dependent [62]. Furthermore, knock-down of ASIC1 expression in D54-MG and in human glioblastoma multiforme U251-MG cells inhibited their migration at physiological pH_o [63,64]. These results imply that ASIC1 channels were active at an extracellular pH that should have been too alkaline to allow significant ASIC1 activation [59], suggesting that the channels must have been activated by other stimuli or sensitised to lower extracellular proton concentrations. There are also reports of acidosis-mediated effects of ASICs in glioblastoma. C6 glioma cells

responded to acid-activation of ASIC1a with apoptotic cell death [65] via increases in intracellular Ca^{2+} concentration [66].

In breast cancer cells, ASIC1 mediated acidosis-dependent cell invasion, tumour growth and lung metastasis [67], and its expression correlates with poor survival in breast cancer patients [67]. In prostate cancer cell lines, ASIC1 knock-down suppressed acidosis-mediated cell invasion and inhibited cell growth in androgen-free medium, hence implicating ASIC1 in castration resistance [68].

In colorectal cancer cells, ASIC2 was upregulated upon acid exposure, and ASIC2 expression levels correlated with cell proliferation [69]. ASIC2 was also implicated in mediating acidosis-dependent invasion of colorectal cancer cells in vitro, which was dependent on activation of the calcineurin-NFAT pathway, and liver metastasis in vivo, and its expression correlated with poor prognosis in colorectal cancer patients [69]. Furthermore, aberrant methylation may contribute to dysregulated ASIC2 expression in glioma cell lines and tumour tissues [70].

5.2. Transient receptor potential (TRP) channels

TRP channels form a superfamily of non-selective cation channels with varying degrees of Ca^{2+} permeability that respond to a variety of distinct stimuli, including chemicals, temperature and mechanical stress [71].

5.2.1. TRPA1

TRPA1 belongs to the ankyrin-family of TRP channels and was recently identified as a mechanosensitive channel protein that opens in response to membrane stretch [72,73]. Intriguingly, human but not rodent or non-human primate TRPA1 also functions as a proton-activated ion channel that starts to respond at pH_o7.0 and peaks around pH_o5.4 [74]. Other cancer-related chemicals that can activate TRPA1 include DNA-damaging electrophiles, cigarette smoke [75] and reactive oxygen species (ROS) [76,77]. This suggests that TRPA1 activation may not only promote cancer progression but may in fact be an early event in cancer development. In agreement with this, TRPA1-mediated Ca^{2+} influx into cells protected these from apoptosis and anoikis as well as reduced their chemosensitivity [78].

TRPA1 expression levels are markedly increased in a variety of cancers compared with healthy tissues, and especially so in cancers of the kidney, lung, breast, head and neck [75,78,79]; upregulation of TRPA1 correlates with unfavourable prognosticators in patients affected by nasopharyngeal carcinoma [80]. In small cell lung cancer (SCL) cells, TRPA1 activation by volatile substances enhanced cell survival by inhibiting apoptosis following serum starvation and promoting anchorage-independent growth of SCL cells, and TRPA1 was more highly expressed in cancerous than healthy lung tissue [81]. In human distal lung epithelial A549 cells, activation of TRPA1 by methyl syringate suppressed hypoxia-induced cyclooxygenase-2 protein production and reduced cell migration and invasion as well as inhibited vascular entothelial growth factor (VEGF) release [82]. In the same cells, pro-inflammatory Il-1α application increased functional TRPA1 expression at the cell surface in an Erk-dependent manner, suggesting that immune cell activity in cancers may promote TRPA1 activation in cancer cells [83]. A recent study has suggested that TRPA1 promotes lung adenocarcinoma progression and metastasis by binding to fibroblast growth factor receptor 2 (FGFR2) via its ankyrin repeat [84]. Ankyrin repeats, a motif associated with protein-protein interactions [85], also convey mechanosensing capabilities to proteins including TRPA1 channels [86,87]. In this context it would be important to establish whether mechanical cues can affect TRPA1-FGFR2 binding positively or negatively. TRPA1 has also been associated with VEGF secretion in human prostate cancer stromal cells, which intriguingly are the only cells in pancreatic cancer tissue to express TRPA1 [88].

5.2.2. TRPV1

TRPV1 was the first TRP channel of the vanilloid family to be reported to be both potentiated and directly activated by extracellular protons, and it was also identified as mechanosensitive channel that responds to cell shrinkage in response to hypertonic challenge [89]. TRPV1 has been investigated in a range of different cancer cells where it can promote a plethora of distinct responses [90].

In many if not most studies, effects of TRPV1 activation is studied by capsaicin application, which activates the channel directly. This is an intriguing point since the osmosensitive TRPV1 channel lacks the first ~ 300 amino acids on the N-terminus, which renders TRPV1 insensitive to capsaicin [89,91]. Therefore the question has to be asked whether osmosensing TRPV1 plays a significant role in cancer progression and this receptor will therefore not be further considered for the purpose of this review.

5.2.3. TRPV4

TRPV4 channels have been reported to be sensitive to a variety of distinct stimuli, including osmotically induced cell swelling, fluid shear, and acidic pH_o [92]. Cell swelling in the absence of osmotic gradients also causes TRPV4 activation, and TRPV4 should therefore be considered a volume- rather than osmo-sensitive channel [93]. Cell volume changes are usually an inevitable consequence of osmotic gradients across the cell membrane; however, extent of cell swelling may be determined not only by the size of the ionic gradient but also by the stiffness of the ECM that may oppose cell swelling (e.g. [94]). Hence, this is a valuable distinction to make as ionic strength can affect protein structure and function independently of cell volume changes in response to osmotic challenge [95]. Exactly how mechanical stimuli lead to activation of TRPV4 is still unclear. Possible mechanisms include TRPV4 being directly activated by membrane stretch [96], but TRPV4 activation mechanisms following cell swelling may indeed vary between cell types [97]. Similarly, whilst it is well established that TRPV4 is activated under conditions of extracellular acidosis, with activation starting around pH_o6 and peaking at pH_o4 [98], the amino acid residue (s) responsible for proton sensing on TRPV4 remain elusive.

In the context of cancer, the sensitivity of TRPV4 to cell volume changes and its activation to reduce cell swelling may not be a desired event because cell swelling can be required to counter compressive forces, and hence TRPV4 activation in response to cell swelling may, counterintuitively, contribute to cell death.

TRPV4 has also been implicated in fluid shear sensing in endothelial cells and is critical for vascular function [99]. Intriguingly, changes in TRPV4 expression have been observed in endothelial cells derived from tumours, and this correlated with altered barrier function, vessel density and diameter, endothelial cell migration, modified pericyte coverage of vessels and tumour growth [100–103]. As discussed, fluid shear is different in tumour vessels compared with normal vessels, and it would be interesting to investigate whether this contributed to aberrant TRPV4 signalling in solid tumours.

TRPV4 has also been implicated in transformed cell function. TRPV4 expression levels are significantly up- or downregulated in a cancer-specific manner compared with the respective healthy control tissue [79,104], suggesting that it fulfils different roles in different cancer types. Even for a given cancer type, activation of TRPV4 has different effect on the cell in which it is expressed. In breast cancer of the basal molecular type, TRPV4 expression was upregulated [105,106]. One study showed that, in these cancers, TRPV4 enhanced metastatic potential by promoting cancer cell softness, blebbing and actin reorganisation [105], while another one reported that pharmacological activation of TRPV4 caused pronounced cell death and reduced tumour growth in vivo [106]. These two studies used different breast cancer cell lines, which likely accounts at least in part for the differences observed.

5.3. Proton-sensing Gq protein coupled receptors

Ovarian Cancer Gene Receptor 1 (OGR1, aka GPR68) is one of three proton-sensing G protein coupled receptors (GPCRs) [107] that signals via G_q and hence intracellular Ca^{2+} rises, mediated by both Ca^{2+} release from intracellular Ca^{2+} stores and Ca^{2+} influx through Ca^{2+} permeable plasma membrane ion channels [108], such as proton-potentiated canonical TRPC4 channels [109,110]. Increases in cAMP concentration and changes in Rho activity following OGR1 stimulation have also been reported [111–114], and it appears that allosteric modulators can influence its G protein preference [115]. OGR1 is inactive at pH_o7.8 and above, partially active at physiological pH_o, and fully active between pH_o6.8 and 6 [111,116,117]. Five histidine residues have been implicated in proton-sensing for OGR1, and these residues are highly preserved in the other members of the proton-sensing GPCR family [107].

The idea that OGR1 is also mechanosensitive was only recently introduced by a study showing that OGR1 is expressed in a subset of endothelia, and that fluid shear could potentiate OGR1-dependent intracellular Ca^{2+} rises with a bell-shaped pH_o dependence that peaked between pH_o7.5 and 6.5 [118]. Furthermore, OGR1 knock-out mice displayed reduced acute flow-mediated dilation and chronic flow-mediated outward remodelling in mesenteric arterioles [118], demonstrating a physiological role for mechanosensing through OGR1.

Another report subsequently showed that OGR1 is sensitive to membrane-stretch by studying OGR1 activity in response to seeding cells on substrates of different stiffness as well as in response to continuous and acute membrane stretch [111]. These experiments revealed that membrane stretch-mediated actin polymerisation is an absolute requirement for OGR1 activation, alongside sufficiently high extracellular proton concentration. Hence, OGR1 functions as coincidence detector of membrane stretch and pH_o, and the level of OGR1 activity is dependent on both extent of membrane stretch and extracellular acidification [111]. The previous study had concluded that fluid shear was not necessary for OGR1 activation [118]; however, all experiments monitoring OGR1 activity had been carried out on cells seeded on hard substrates that promote membrane stretch and hence OGR1 activation. Hence, it is unclear whether OGR1 activation is membrane stretch-dependent or whether other mechanical stressors (shear, compression) can substitute for it.

As the name indicates, OGR1 was originally cloned from ovarian cancer tissue (but is absent in healthy ovarian tissue) [119], and was shown to activate gene transcription pathways in response to Ca^{2+} release from intracellular Ca^{2+} stores, thus linking extracellular acidosis to changes in gene transcription [108]. OGR1 activation by low pH_o has also been linked to gene transcription via the mechanosensitive transcription factor Yes-Associated Protein (YAP) that has been implicated in cancer progression [120–122]. Intriguingly, OGR1 expression can be increased under hypoxic conditions at low pH_o [123], i.e. under conditions likely to be encountered in solid tumours. Furthermore, OGR1 itself can influence intra- and extracellular pH by modulating the activity of plasmalemmal sodium proton exchangers (NHE) and H^+-ATPases [124]. Taken together, all this suggests that OGR1 activation will occur and increase during cancer development and progression, and that OGR1 activity has the potential to enhance the pathological phenotype.

Accordingly, OGR1 has been found and implicated in a variety of cancers [123,125–127], and whether it supports or inhibits tumour growth appears to depend on the cell and cancer type in which it is expressed. For example, in CAFs, OGR1 promotes activation of mesenchymal stem cells [120], and pancreatic cancer cells increase OGR1 expression in co-cultured CAFs, resulting in increased production of pro-oncogenic Il-6 as well as enhanced fibrotic marker expression in these cells [114]. This is consistent with OGR1 being linked to fibrogenesis [114,128]. Furthermore, OGR1-expression in CAFs in tumour stroma spheroids supports spheroid formation and maintenance, as well as Il-6 and -8 secretion [129]. In human cancers, OGR1 is found to be expressed by cancer-associated myeloid and CD3+ T-cells [129–131] and CAFs [125,129], and implantation of cancer cells into OGR1 knock-out mice resulted in reduced tumour growth in all models tested [129–131].

All of this suggests that OGR1 expression in cancer-supporting cells promotes cancer progression. This is in contrast with OGR1 expression in the actual cancer cells. OGR1 activation leads to inhibition of migration in a variety of cells, including cancer cells [110,112,132]. Furthermore, OGR1 inhibits ovarian cancer cell proliferation whilst increasing cell adhesion to the ECM [132], and OGR1 is more highly expressed in non-metastatic than metastatic prostate cancer [133]. OGR1 suppresses migration of prostate cancer cells, and OGR1-overepxressing prostate cancer cells, when implanted in mice, generate fewer metastases than their OGR1-negative counterparts [133]. Intriguingly, primary tumour growth was unaffected by OGR1 overexpression, implying a correlation between migration inhibition through OGR1 and the ability for metastasis [133].

As mentioned above, OGR1 is one of three proton-sensing GPCRs. To date, it is unclear whether the other two members (GPR4 and GPR65, aka TDAG8 [107]), are also mechano-sensitive. These two receptors couple via G_s (107), and it will be important to establish if only the predominantly G_q-coupled proton-sensing GPCR OGR1 is also mechano-sensitive.

It is noteworthy that OGR1 has been shown to be crucial for enamel formation in humans [134] and bone metabolism in rodents [135–137]. The finding that OGR1 is required for enamel formation is an excellent illustration for a role of OGR1 in shaping the extracellular matrix of tissues. Furthermore, bones and teeth are the hardest tissues in the human body and experience changes in extracellular pH, and it is therefore not surprising that lack of OGR1 function should affect these tissues in particular since one would expect OGR1 to be acutely sensitive to pH changes in these hard environments.

6. Conclusions

Different studies use different means to investigate a role for a particular protein in cancer, ranging from pharmacological activation of proteins in cancer cell lines in vitro to in vivo experiments in which cancer cells are injected into animals, and cancer growth and metastasis is monitored under conditions of genetic knock-down of the protein of interest in either the host animal or injected cell line.

For mechanosensitive proteins, the physical environment is of utmost importance, and it is easy to forget that cells seeded on plastic or glass surfaces experience an environment that is a mechanical challenge in its own right. Therefore, constitutive activity of a protein (as described for ASIC1 in high-grade glioma cultured cells) could reflect its activation, or its sensitisation to other stimuli that might then trigger activation of the protein at levels that are much lower than normally required. In vivo experiments, in which human cancer cell lines are injected into mice, are equally difficult to interpret since it is unlikely that tissue mechanic properties of mice match those of humans, and this is further complicated when cells are not injected orthotopically.

Equally, use of pharmacological agonists that are not endogenously produced by organisms always risks activating proteins in a manner distinct from the physiological agonist (e.g. G protein coupling, desensitisation kinetics, conductance, mean open time). This is particularly important for proteins causing changes in intracellular Ca^{2+} levels because different spatio-temporal patterns in Ca^{2+} signals will give rise to distinct cellular responses.

Accordingly, investigating polymodal proteins such as mechano- and proton-sensitive receptors and ion channels discussed here, is even more complicated. Whether extracellular protons and mechanical stress activate a particular protein independently, or whether activation via one mechanism affects (further) stimulation via the other (be it synergistically or antagonistically) is likely to depend on the protein

under investigation. To date, only OGR1 has been shown to be critically dependent on both mechanical and proton stimulation for function. Even under conditions of strong acidosis (pH_o6.0), OGR1 is not significantly active unless it also experiences membrane stretch, and vice versa. Hence, OGR1 will lay "dormant" in the presence of only one of the two stimuli, and it will respond more strongly the greater the two stimuli are. In contrast, ASIC2 and 3 can only function as either proton- or mechano-sensors, but not both at the same time. As described above, their ability to respond to a mechanical stimulus depends on the presence of an additional protein, and interaction with this protein renders ASIC2 and 3 mechanosensitive but proton-insensitive. Hence, it is not the presence of the mechanical stimulus that prevents proton-sensing but the presence of another protein.

For TRPA1 and TRPV4, it is unclear whether mechano- and proton-sensing are independent processes that can take place at the same time and have additive effects on channel activity, or whether they interfere with each other (e.g. desensitising or sensitising the channel protein to the other stimulus, or changing the quality of the response). This will be important to elucidates, as it will help establish whether convergence of two stimuli on one protein can lead to different outcomes depending on how the protein integrates the stimuli, ranging from both stimuli acting synergistically (OGR1), to (conceivably) one stimulus inhibiting the detection of the other. That way, different signalling events and cell behaviours can be triggered in response to the same (combination of) stimuli.

How the microenvironment influences cancer progression is a pertinent question, and proteins able to sense two environmental parameters commonly dysregulated in solid cancers are likely to play key roles in this process. A better understanding of how these two cues come together at the protein level, and what impact they have on intracellular Ca^{2+} signals and subsequent downstream signalling cascades will enable us to make better predictions of likely outcomes of protein stimulation, and hopefully will enable us to use expression (patterns) of these proteins for diagnostic and therapeutic purposes in the future.

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