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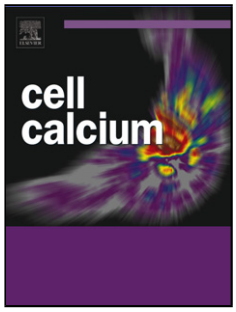

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ORAI channels and cancer

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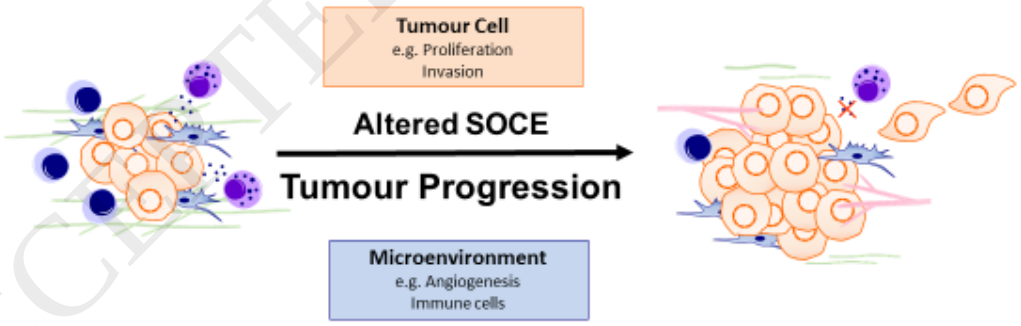
Graphical abstract



Highlights

- This review provides an overview of ORAI channels in cancer

- The expression of ORAI isoforms is remodelled in many cancer types and subtypes
- ORAI channels have been linked to cancer cell proliferation and invasiveness
- ORAI channels are important in events that occur in the tumour microenvironment

Abstract

Cancer is a major cause of death. The diversity of cancer types and the propensity of cancers to acquire resistance to therapies, including new molecularly targeted and immune-based therapies, drives the search for new ways to understand cancer progression. The remodelling of calcium (Ca^{2+}) signalling and the role of the Ca^{2+} signal in controlling key events in cancer cells such as proliferation, invasion and the acquisition of resistance to cell death pathways is well established. Most of the work defining such changes has focused on Ca^{2+} permeable Transient Receptor Potential (TRP) Channels and some voltage gated Ca^{2+} channels. However, the identification of ORAI channels, a little more than a decade ago, has added a new dimension to how a Ca^{2+} influx pathway can be remodelled in some cancers and also how calcium signalling could contribute to tumour progression. ORAI Ca^{2+} channels are now an exemplar for how changes in the expression of specific isoforms of a Ca^{2+} channel component can occur in cancer, and how such changes can vary between cancer types (e.g. breast cancer versus prostate cancer), and even subtypes (e.g. oestrogen receptor positive versus oestrogen receptor negative breast cancers). ORAI channels and store operated Ca^{2+} entry are also highlighting the diverse roles of Ca^{2+} influx pathways in events such as the growth and metastasis of cancers, the development of therapeutic resistance and the contribution of tumour

microenvironmental factors in cancer progression. In this review we will highlight some of the studies that have provided evidence for the need to deepen our understanding of ORAI Ca^{2+} channels in cancer. Many of these studies have also suggested new ways on how we can exploit the role of ORAI channels in cancer relevant processes to develop or inform new therapeutic strategies.

Keywords: Cancer; Calcium; ORAI; STIM

1.0 Introduction

As noted throughout this special issue, the identification of the molecular components of store operated calcium (Ca^{2+}) entry (SOCE), has revolutionized not just the field of Ca^{2+}signalling but it has also provided new insights into many diseases. Studies of ORAI channels in cancer have mostly focused on ORAI1, the canonical channel component for SOCE identified in 2006[1-3]. In this context, ORAI1 is activated by the endoplasmic Ca^{2+}sensor stromal interaction molecule 1 (STIM1) upon Ca^{2+} store depletion, promoting Ca^{2+} influx for Ca^{2+} store refilling and/or the activation of key Ca^{2+}dependent processes. ORAI1 is structurally very different to other Ca^{2+} channels except for its related isoforms ORAI2 and ORAI3[1], the latter of which is only found in mammals[4]. The contribution of ORAI2 and ORAI3 to SOCE may be context dependent, such as the negative fine-tuning role of Orai2 through heteromeric Orai1/Orai2 channels in mouse T-cells[5]. ORAI2 and ORAI3 have also been proposed to make contributions directly to Ca^{2+} influx in response to specific factors [4]. ORAI isoforms are found ubiquitously but some cell types appear to have higher levels of specific isoforms such as ORAI1 in immune

cells and ORAI2 in the brain[7-9]. ORAI isoforms may respond differently to stimuli and have distinct roles, their changes in cancer and their contribution to cancer relevant processes is likely to be similarly diverse.

The impact of the discovery of Ca^{2+} channels in oncology is evident from the approximately 200 PubMed listed publications related to channels in cancer, including specific reviews on the contribution of channels to tumour progression[10-13]. It is beyond the scope of this review to explore all of these contributions to our understanding of ORAI channels in cancer. Here, we have sought to provide a general overview of ORAI Ca^{2+} channels in cancer. We particularly focus on what we regard as three key aspects of ORAI channels in cancer; 1) the remodelling of ORAI channels in different cancers, 2) ORAI Ca^{2+} channels in the transformed cell and 3) the contribution of ORAI channels in cells relevant to the tumour microenvironment.

2.0 ORAI channel remodelling in cancer cells

Mutations in genes are a defining feature of cancer and many are drivers of the oncogenic phenotype and/or are contributors of tumour progression through promotion of cancer cell proliferation, metastasis or resistance to death signals[14]. Indeed, the diversity of gene mutations can change during cancer progression, or at metastatic sites[15]. One could imagine that mutations in some Ca^{2+} channels that increase channel activity could promote metastatic or proliferative pathways, or those that would reduce activity could potentially bestow resistance to apoptotic signals. ORAI1 mutations have been reported to occur in some cancers from the cBioPortal database and some of these mutations have been shown to confer

constitutive activity to ORAI1 channels[16]. These activating mutations were found in cancers from patients with colorectal, stomach and uterine cancer. Although such changes could potentially promote tumour progression through activation of proliferative and/or metastatic signalling pathways, so far, such mutations appear to be very rare events for ORAI1[16] compared to mutations in other genes, including those that have strong links to some cancers such as mutations in p53 or RAS[14]. However, there may be cases, as recently suggested, where mutations may remodel SOCE, e.g. oncogenic KRAS induced changes in STIM1 expression via ERK signalling[17]. However, changes in a specific Ca^{2+} influx pathway via an ORAI channel is unlikely to be a driver of transformation and ORAI channels are unlikely to ever be classified as oncogenes[10]. Nevertheless, a remodelling of Ca^{2+} influx through ORAI Ca^{2+} channels may impart features that promote disease progression, such as the promotion of growth and invasiveness or reduced sensitivity to apoptotic stimuli. Such changes have been proposed to provide opportunities for therapeutic exploitation. Indeed, Rhizen Pharmaceuticals have recently reported the commencement of a human phase 1/1B clinical trial of an ORAI1 inhibitor for the eventual treatment of relapsed or refractory Non-Hodgkin Lymphoma (ClinicalTrials.gov (NCT03119467)). In this section, we will discuss examples of the types of ORAI Ca^{2+} channel alterations that have been reported in some cancers. We will particularly highlight how such changes can be cancer type or even subtype dependent, and (in some cases), even be linked to disease outcomes and survival.

ORAI isoforms and STIM1 and STIM2 expression changes have been extensively evaluated in a number of cancer focused studies. Changes in ORAI channel expression could contribute to the promotion of proliferative or metastatic pathways

or enhance the ability of cancer cells to avoid cell death. Although links between expression and tumour progression have not been established in every case, changes in expression of ORAI channels, STIM1, and STIM2 are evident in many studies of human clinical samples (Table 1). Indeed, recent analysis of glioblastoma RNA-sequencing data in The Cancer Genome Atlas has found higher STIM1 expression is correlated with poor survival[18].

As will be seen throughout this review, there are often examples where the contributions of ORAI Ca^{2+} channels are dependent on the type or even subtype of cancer, as well as the specific ORAI or STIM isoform. This is the case for alterations of expression in cancer and is particularly exemplified in studies assessing ORAI channel levels in breast cancer cells. Breast cancer is arguably the cancer type where diversity in gene expression and drug target expression has been the most comprehensively defined and where such differences have the greatest impact on treatment. For example, women whose breast cancers express the oestrogen receptor will often be treated with anti-oestrogen therapies such as tamoxifen and those whose cancers overexpress human epidermal growth factor receptor 2 (HER2) may receive agents such as trastuzumab[19]. Breast cancers that do not overexpress HER2 or the oestrogen and progesterone receptors are classified as triple negative breast cancer (TNBC), these cancers overlap somewhat with the molecularly defined basal molecular breast cancer subtype[19]. TNBCs lack current molecularly targeted therapies and the assessment and development of such agents is an identified priority to improve patient outcomes[19]. In the context of ORAI channels, elevated ORAI1 seems to be a feature of basal breast cancers compared to non-basal as identified in clinical samples[20]. In contrast, cell line studies strongly

suggest that ORAI3 is elevated in oestrogen receptor positive breast cancer cells[6]. Indeed, ORAI3 is a regulator of SOCE in MCF-7 oestrogen receptor positive breast cancer cells, but not in the basal triple negative MDA-MB-231 breast cancer cell line[6]. This is an example where changes in ORAI channel expression appears to be directly linked to a major functional change in the nature of Ca^{2+} influx, i.e. the actual ORAI isoforms that contribute to SOCE in a breast cancer cell. The association between ORAI3 and oestrogen receptor status of breast cancer cells is also reflected by the ability of oestrogen receptor α silencing to reduce ORAI3 but not ORAI1 expression levels in MCF-7 breast cancer cells[21]. Elevation of ORAI3 has also been reported in breast cancer clinical samples[22]. Further work on clinical samples is still required to fully define the association between specific breast cancer subtypes and ORAI isoform expression levels. Another element of breast cancer subtype differences in the potential remodelling of ORAI-mediated Ca^{2+} influx is seen in the canonical ORAI1 activators STIM1 and STIM2. In breast cancer of the basal molecular subtype but not other molecular subtypes (HER2, Luminal A, Luminal B) samples are more often associated with high STIM1 and low STIM2 levels[20].

As illustrated in Table 1, there has now been a variety of studies that demonstrate the overexpression of ORAI channels in clinical samples of other cancer types. For example, ORAI1 levels are elevated in cancer of the liver[23], osesophagus [24], stomach[25] and in renal cancer[26]. ORAI3 is elevated in lung cancer[27, 28]. In vitro studies have also provided clues to potential changes in expression in cancer, such as high levels of ORAI2 in an acute myeloid leukaemia cell line[29]. In some cases the changes in ORAI channel isoform expression includes a down regulation that may, as described below, contribute to resistance to apoptotic pathways. This is

exemplified by the down regulation of ORAI1 in some prostate cancers that have developed castrate resistance[30]. There are also some cases where the relative levels of ORAI isoforms may be a feature of a cancer cell. A specific example is seen in the relationship between ORAI1 and ORAI3 levels; it has been reported that there is a formation of ORAI1/3 heteromeric channels in some prostate cancers proposed to be driven by an upregulation of ORAI3[31]. However, further studies are still required to conclusively demonstrate the direct formation of ORAI heteromeric channels and to define their specific roles in prostate cancer and other diseases. The expression of ORAI channels may also be dynamic in cancer cells and influenced by tumour microenvironmental factors such as growth factors and hypoxia[32-34]. STIM1 appears important in the promotion of hepatocarcinogenesis by hypoxia; hypoxia-inducible factor-1 alpha (HIF-1α) promotes STIM1 expression in hepatocarcinoma cells and there is a positive correlation between STIM1 and HIF-1α levels in liver cancer clinical samples [35]. In some cases alterations in the expression levels of another protein may be significant in the context of ORAI channels as is the case for SigmaR1 (a stress-activated chaperone). SigmaR1 is overexpressed in some colorectal cancers and appears to promote Ca^{2+} influx via ORAI1 through promoting the interaction between ORAI1 and the Ca^{2+} activated potassium channel - SK3[36].The consequence of this interaction is altered Ca^{2+}signalling and the promotion of Ca^{2+} dependent processes that regulate cell migration. Indeed silencing of SigmaR1 supresses SOCE in HCT-116 colon cancer cells[36]. Hence, potential remodelling of ORAI-mediated Ca^{2+} signalling in cancer cells may go beyond changes in expression of SOCE components and their related isoforms.

Differences in ORAI channel levels *per se* do not indicate that an ORAI channel isoform is important in processes in a cancer cell or even that there is a change in Ca^{2+} influx. For example, changes in mRNA may not be reflected in protein levels or trafficking changes may lead to no significant alteration in a Ca^{2+} influx pathway. Indeed, alterations in ORAI channel trafficking could itself contribute to a remodelling of Ca^{2+} signalling in a cancer cell. This is an area which has received little attention in cancer studies. Hence, expression levels alone are insufficient to draw conclusions regarding ORAI channels as a potential drug target for a specific cancer type and/or to even predict a contribution to a tumourigenic pathway. However, there are studies that have defined changes in ORAI mediated Ca^{2+} influx in cancer cells. These include the aforementioned differences between oestrogen receptor positive and oestrogen receptor negative breast cancer cells in the ability of ORAI3 to contribute to SOCE[6], the role of ORAI3 levels in the response of prostate cancer cells to arachidonic acid activated Ca^{2+} influx[31], and the presence of ORAI1 mediated Ca^{2+} oscillations in oesophageal cancer cells that are absent in non-tumourous cells[24]. There are cases where changes in SOCE are likely to be subtype dependent, as exemplified in melanoma cells. Melanoma cells with enhancement of Wnt5A (a metastatic driver) appear to have reduced SOCE, whereas other melanoma cells may have enhanced SOCE[37-39]. Although this diversity may be a potential challenge to targeting SOCE in melanoma, a case has been made that the “normalizing” of SOCE may be the desired outcome, rather than simple inhibition or activation[37]. It is tempting to point to examples where levels of ORAI or STIM isoforms are correlated with prognosis as evidence of the significance of ORAI channel levels in tumour progression pathways. Examples of the potential association between ORAI or STIM isoform levels and prognosis include the

association with high levels of ORAI3 with greater metastasis and poorer survival in patients with resectable lung adenocarcinoma[27], decreased time to recurrence and lower overall survival in gastric cancers with high ORAI1 and STIM1 expression[25], and a correlation of high STIM1 levels with tumour size and lymph node metastases in cervical cancer[40]. However, such associations between expression and survival could simply be correlative; ORAI channel and STIM isoform levels may be a marker for a subtype associated with a poorer clinical outcome without itself contributing to disease progression. For example, breast cancers with high STIM1 and low STIM2 levels are associated with poor survival but this is also a feature of the basal molecular subtype that often have poorer prognosis than some other molecular subtypes (e.g. luminal A)[20]. Alternatively, the altered expression of an ORAI or STIM isoform may be secondary to another pathway that does contribute to disease progression. However, evidence that the significance of ORAI channels in cancer goes beyond simple changes in expression is seen in the ability of silencing and/or pharmacological modulation of ORAI channel mediated Ca^{2+} influx to alter many of the classic hallmarks of cancer pathways. Such changes include effects on cancer cell proliferation and invasiveness and are discussed in the section below.

3.0 ORAI calcium channels in cancer cells and their regulation of events important in tumour progression

A variety of studies through gene silencing and in some cases pharmacological approaches have provided compelling evidence for the role of ORAI channels in events essential for tumour progression, including proliferation, metastasis, invasion, resistance to cell death and the development of resistance to therapies (Figure 1).

Studies have also shown that overexpression of components of SOCE are sufficient to bestow tumorigenic features in non-tumorigenic cells, for example ORAI1 and STIM1 overexpression increases the migration and invasiveness of MCF-10A breast cells[41]. Below we highlight some examples of how ORAI channel isoforms in cancer cells appear to promote tumour processes and, where possible, describe example mechanisms. In many cases, contributions and mechanisms may differ greatly between cancer types and subtypes. The discussion below should be viewed in this context. We also note that there are opportunities to enhance our understanding of the roles of ORAI isoforms and STIM1 and STIM2 in cancer through the use of in vivo models that go beyond xenografts in immunodeficient mice. Such models include syngeneic mouse models[42] (which has only been used in a limited number studies in this field to probe immune system involvement[43]), patient derived xenografts, and genetically engineered mouse cancer models[42]. An example of how the use of more diverse models and approaches could provide new insights into the roles of ORAI isoforms and STIM1 and STIM2 in tumour progression is reflected in studies of the Ca^{2+} pump PMCA2 in breast cancer[44]. Mice harbouring a null mutation in the Pmca2 gene (Dfw-2J) crossed with a transgenic mouse model of HER2 overexpression (MMTV-Neu) show reduced tumour incidence and prolonged tumour latency compared to MMTV-Neu mice with normal Pmca2 expression levels[44].

3.1 ORAI channels and cancer cell proliferation

Studies have identified in a variety of cancer relevant in vitro and in vivo models the ability of ORAI channels to contribute to cancer cell growth and proliferation. One of

the earliest studies of ORAI1 in cancer proliferation occurred in breast cancer. Silencing of ORAI1 in the human breast cell line MCF-7 reduces proliferation and anchorage independent growth in vitro and tumour burden in vivo[45]. Similar effects have been reported with ORAI3 silencing with reductions in anchorage independent growth in vitro and tumour size in vivo[21]. These effects of ORAI3 silencing in MCF-7 cells are associated with attenuation of the activity of ERK1/2, focal adhesion kinase, transcriptional activity of nuclear factor for activated T cells (NFAT)[21] and C-MYC[46]. Although ORAI2 mRNA is detected in breast cancer cell lines and may even be elevated in some specific breast cancer cell lines[20], there has been no comprehensive studies of the role of ORAI2 in breast cancer cell proliferation or other tumourigenic pathways in breast cancer cells. However, as ORAI2 silencing in HL60 (an acute myeloid leukaemia cell line[29]) reduces their proliferation rates, further studies of ORAI2 in proliferative pathways in other cancer cell line models and in in vivo models would seem to be a priority.

In addition to being elevated in oesophageal squamous cell carcinoma[24], ORAI1 appears to be critical in maintaining elevated proliferation rates in these cells[24, 47]. ORAI1 also appears to be important in the anti-proliferative effects of Zn^{2+} on oesophageal squamous cell carcinoma cell proliferation[47]. This may have clinical significance given the association between zinc deficiency and oesophageal squamous cell cancer[47, 48]. Other examples of cancer cell models where ORAI1 has been implicated in proliferation includes gastric cancer[25], rhabdomyosarcoma[49], and clear cell renal carcinoma[26]. In many cases these effects may be due to a remodelling of how cancer cells respond to key growth

factors. Such is the case in colon cancer cells, where ORAI1 silencing attenuates the ability of epidermal growth factor to induce increases in COX-2[50].

In the context of ORAI3, in addition to roles in the proliferation of oestrogen positive breast cancer cells lines as discussed above, ORAI3 is also a regulator of the proliferation of small cell lung adenocarcinoma cells[28]. In prostate cancer, enhanced ORAI3 expression promotes the formation of ORAI1/3 heteromeric channels that appears, via NFAT, to promote arachidonic acid activation of cancer cell proliferation[31]. It should be noted that ORAI channels are not global regulators of cancer cell proliferation as exemplified in a study in human glioblastoma[51], where ORAI1 and STIM1 silencing had little to no effect on proliferation, but, as discussed below did attenuate invasive pathways[51].

3.2 ORAI channels and cancer cell migration and invasiveness

ORAI1 has been linked to a variety of processes in cancer cells important in metastasis, such as cell motility during cancer cell migration and also events important in invasion, such as interactions and modification of the extracellular matrix. One of the first links with ORAI1 and metastasis was observed in MDA-MB-231 breast cancer cells, where silencing of ORAI1 reduced serum induced cell migration, invasion through matrigel and the establishment of metastasis in vivo[41]. These effects were mediated in part through changes in focal adhesion molecule turn over[41]. In MDA-MB-231 breast cancer cells, ORAI1 is also important in the translocation and release of enolase-1 that regulates the invasion process[52]. This adds a further dimension to how ORAI1 may influence breast cancer metastasis.

ORAI1 is also important in metastatic progression in other cancers. For example, oscillations in intracellular Ca^{2+} levels that are dependent on ORAI1, appear to be critical in the migration of WM793 melanoma cells[53]. ORAI1 silencing attenuates invadopodium formation via SRC in this model[53]. ORAI1 silencing is able to inhibit MT1–matrix metalloproteinase (MMP) recycling to the plasma membrane and hence inhibit degradation of the extracellular matrix required for invasion[53]. Another example of the role of ORAI1 in cancer invasiveness is seen in glioblastoma. Despite having a negligible effect on the proliferation of primary glioblastoma cells, ORAI1 silencing greatly reduces serum activation of invasion through matrigel[51]. In contrast ORAI1 silencing has no significant effect on the invasive properties of human primary astrocytes using the same assay[51] implicating that ORAI1 mediated SOCE might be particularly important in processes required the invasion of glioblastoma cells. There has been less extensive direct investigation of ORAI3 and ORAI2 in the context of processes important in cancer metastasis. However, ORAI3 silencing supresses the in vitro matrigel invasiveness of MCF-7 breast cancer cells[21], and it will be interesting for future studies to assess the consequence of ORAI3 silencing on metastasis in vivo with a more invasive ORAI3 overexpressing breast cancer cell line. ORAI3 silencing also attenuates arachidonic acid promotion of migration in an in vitro model of gastro-enteropancreatic neuroendocrine tumours[54]. In the context of ORAI2, silencing of this ORAI isoform in an acute myeloid leukemia cell line (HL60), reduces transwell migration in a manner that may be related to the effects of ORAI2 silencing on the FAK phosphorylation[29]. Although this may not be a feature of all models and cancer types[55], there is a report of ORAI1 being potentially important in the acquisition of a more invasive phenotype, through effects on epithelial to mesenchymal transition. For example,

silencing of ORAI1 reduces levels of the mesenchymal markers vimentin and fibronectin as well as migration rates in human gastric cancer cells[25]. In lung adenocarcinoma cells lines ORAI1 silencing also reduces epithelial to mesenchymal transition marker induction induced by fibroblast growth factor 4[56]. Studies assessing the remodelling of Ca^{2+} influx via ORAI channels and the roles of this Ca^{2+} influx in cancer cells adopting a more invasive state should now continue with other ORAI isoforms and in other models.

3.3 ORAI channels and cancer cell death

The Ca^{2+} signal has been related to a variety of cell death pathways in a variety of cell types including neurons. Indeed, the nature of Ca^{2+} signal changes has been related to the type of death. Excessive declines or increases in intracellular Ca^{2+} levels are capable of reducing cell viability. It is therefore not surprising that studies of ORAI Ca^{2+} channels in the death of cancer cells can be diverse and complex.

Like almost all other aspects of the study of ORAI channels, assessment of the role of ORAI channels in cancer cell death have mostly focused on ORAI1. The studies of ORAI1 in the context of cancer cell death do provide the opportunity to reflect on the potential significance of the down regulation of a Ca^{2+} channel as a way for a cancer cell to remodel their Ca^{2+} signalling to avoid cell death. It has been proposed that as prostate cancer progresses towards the more metastatic and therapy resistant androgen independence stage, there is a downregulation of ORAI1 and a depression of SOCE[30, 57]. This down regulation of SOCE in prostate cancer cells may then bestow apoptotic resistance to agents such as tumour necrosis factor α

and cisplatin[57]. Indeed, a number of subsequent studies from a diverse set of researchers have reported the ability of ORAI1 silencing and/or suppression of SOCE (via pharmacology agents or STIM silencing) to reduce induced cell death in a variety of cell types [58-60]. However, increasing SOCE should not always be seen as a way to induce cell death. In some models, SOCE activation by Ca^{2+} store depletion by thapsigargin is not the cause of cell death induced by this agent, but instead is the result of reduced endoplasmic Ca^{2+} levels and unfolded protein responses[61]. There may be cases where ORAI1 silencing and/or SOCE inhibition may promote cancer cell death. In this context, ORAI1 silencing has been reported to be sufficient to induce apoptosis even in the absence of external stimuli in rat glioblastoma cells[62]. Another example of ORAI1 inhibition to potentially promote cancer cell death is seen by the role of SOCE in the induction of CD-95-dependent apoptosis by Rituximab in Non-Hodgkin B Lymphoma cells, where reduced SOCE increases the effectiveness of Rituximab in both in vitro and in vivo models[63]. These later examples provide possible avenues to exploit the overexpression of ORAI1 in some cancers by inhibiting SOCE to promote the death of cancer cells. However, this aspect of the potential targeting of ORAI1 in cancer cells requires further study.

ORAI3 has also been linked to cell death pathways in cancer cells. Somewhat similar to the reports of ORAI1 silencing increasing apoptosis in the absence of external stimuli in rat glioblastoma cells[62], ORAI3 silencing in MCF-7 breast cancer cell (a cell line where ORAI3 contributes to SOCE) induces a higher percentage of apoptotic cells which is associated with an increase in the BAX/BCL-2 ratio[22]. This induction of apoptosis by ORAI3 silencing is not seen in all cancer types associated

with ORAI3, as silencing of ORAI3 does not induce death in non-small cell lung cancer cell lines[28].

3.4 ORAI channels and resistance to therapy

A variety of Ca^{2+} permeable ion channels have been linked to resistance to cancer therapies, either through changes in expression as a consequence of drug exposure and/or their involvement of events critical to drug resistance. This is epitomized by TRPC5. TRPC5 has been shown to be a critical regulator of the upregulation of multi-drug resistance ATPase 1 (also known as p-glycoprotein) as MCF-7 breast cancer cells acquire resistance to doxorubicin[64]. A series of studies have defined the importance of NFATC3 and also extracellular vesicle transfer of TRPC5 from resistant to sensitive cells in the acquisition of this resistance cascade[64, 65]. The value of understanding the role of ion channels in therapeutic resistance mechanisms in cancer is demonstrated by the ability of TRPC5 inhibition to restore sensitivity to doxorubicin in in vivo models of MCF-7 breast cancer cells with resistance[64] and how elevated levels of TRPC5 appear to be associated with the response to therapy in breast cancer patients[65]. By comparison our understanding of ORAI channels as players in and markers of therapeutic resistance is still in its infancy. However, recent work has established a strong link between ORAI3 and resistance in breast cancer. Induced overexpression of ORAI3 in T47D breast cancer cells results in resistance to cisplatin, 5- fluorouracil and paclitaxel[66]. The mechanism for ORAI3 induced resistance to therapies is dependent on p53, since ORAI3 overexpressing breast cancer cells had far less induction of p53 by cell death inducers than their matching controls[66]. Moreover, there is evidence that in a way that is somewhat analogous to TRPC5, higher levels of ORAI3 are associated with a

poorer response to therapy in large scale breast cancer cohorts derived from the NCBI Gene Expression Omnibus [66]. When this recent study is paired with the association between the down regulation of ORAI1 with resistance to apoptotic stimuli in prostate cancer[57] and studies showing that ORAI1 and STIM1 silencing promotes apoptosis induced by the chemotherapeutic agents 5-fluorouracil and gemcitabine in a pancreatic adenocarcinoma cell line (Panc1)[67], it is clear that the assessment of ORAI channels in the context of intrinsic and acquired resistance in cancer therapy in different types of cancers is now a priority. The diversity of resistance pathways associated with cytotoxic as well as current and emerging molecularly targeted therapies provides an array of avenues for investigation.

4.0 ORAI calcium channels in cells of the tumour microenvironment

The tumour microenvironment is an essential component in the regulation and control of tumour progression[68-70]. Below we consider some of these microenvironment pathways and ORAI channels and discuss (as outlined in Figure 2), which areas require more detailed assessment.

4.1 ORAI channels, immune cells and cancer

Immune cells in the tumour microenvironment (Figure 2) play diverse roles in disease progression. The success of recent new immune based therapies demonstrate the powerful potential of the immune system to control tumour progression, yet immune cell inflammatory pathways can also be tumour promoting, via positive effects on growth and metastatic potential[68]. Of course, as previously

noted, there is a clear risk that any cancer therapy that targets ORAI1 may supress anti-tumour immune system pathways (cytotoxic CD8+ T cells & natural killer (NK) cells), since these rely on SOCE[71]. This is supported by the absence of effective cytotoxic T lymphocyte-mediated anti-tumour immunity against the growth of melanoma (B16-Ova) and colon (MC-38) cancer cells in double knockout mice for Stim1 and Stim2[43]. However, recent studies of Zhou et al[72] have led them to postulate that there may be an optimal level of Ca^{2+} influx for inducing the efficient cytotoxic effects of cytotoxic T lymphocytes and NK cells. In contrast to the expected inhibition of activity with complete silencing of ORAI1, partial silencing of ORAI1 in cytotoxic T lymphocytes increased the efficiency of cancer cell killing[72]. Further studies in other in vitro systems and also in in vivo models are required to definitively determined if there is indeed a “sweet spot” for ORAI1 inhibition; one that can supress cancer cell proliferation and/or invasiveness but which would promote (or at least not inhibit) the actions of cytotoxic T lymphocytes and NK cells. Further studies are particularly important given the observation that BTP2 (a pharmacological inhibitor of SOCE) at concentrations that sub-maximally inhibit SOCE, partially inhibits rather than promotes the cytotoxicity of tumour-specific cytotoxic T lymphocytes[43]. Studies with Orai1 knockout in vivo and different cancer cell lines would also add to the studies reported in double knockout mice for Stim1 and Stim2[43]. Further assessment of the recently described Orai2 knockout mouse and the Orai3 knockout mouse, when developed, will enhance our understanding of the potential adverse effects that global inhibition of these two isoforms may have in any future therapies, although the Orai2 knockout animal appears to be devoid of some of the major phenotypes associated with the loss of Orai1[5].

4.2 ORAI channels and the tumour vasculature

The development of new blood vessels is a key aspect of the tumour microenvironment (Figure 2) and is one that is essential for the growth of the primary tumour and in some cases may influence the nature of metastatic progression. Indeed angiogenesis inhibitors are a class of anti-cancer agent[73]. A variety of Ca^{2+} permeable ion channels have been assessed in cancer models in the context of angiogenesis in cancer. One example is TRPV4, the targeting of which in the context of the tumour vasculature is multifaceted. TRPV4 is elevated in endothelial cells derived from breast cancers, is important in the migration of these cells and has been proposed as a target for novel anti-angiogenic agents[74]. Another way to exploit the role of TRPV4 in angiogenesis in tumours is via activation of TRPV4. Activation of TRPV4 can improve the quality of the tumour vasculature and thus improve the ability of cytotoxic drugs to enter the tumour and act on cancer cells[75]. Although ORAI Ca^{2+} channels have not yet been investigated to the same depth in specific cancer models and we therefore do not yet fully know if ORAI channels could be targeted like TRPV4 to influence angiogenesis, there are emerging signs that ORAI channels may play important, perhaps even critical, roles in tumour angiogenesis. Studies using in an vitro model of endothelial cell tube formation and conditioned media from triple negative breast cancer cells, suggested that ORAI1 in triple negative breast cancer cells plays a role in how hypoxia promotes induction of angiogenesis[32]. Similar results have been recently reported by the same group using colon cancer cells (HCT-116 and SW80), where silencing of ORAI1 reduced hypoxia-induced tube formation of HMEC-1 endothelial cells[34]. Overexpression of the SOCE activator STIM1 in cervical cancer cells promotes angiogenesis in vivo

and silencing of STIM1 reduces angiogenesis[40]. Collectively these studies of ORAI1 and STIM1 in tumour angiogenesis suggest that the other isoforms of ORAI should be examined and the assessment of SOCE in the context of the tumour vasculature should continue. Such assessment should particularly focus on pharmacological regulators of SOCE in more diverse in vivo cancer models.

4.3 ORAI channels and other tumour microenvironment factors

The tumour microenvironment is a complex mix of different cell types, growth factors, nutrient and oxygen levels (Figure 2)[68-70]. The recent report that extracellular collagen-1 through Discoidin domain receptor 1 appears to promote the interaction of the potassium channel Kv10.1 and ORAI1 in breast cancer cells to enhance breast cancer survival, is an example of the potentially complex interplay between cancer cells, their microenvironment, ORAI1-mediated influx and other ion channels[76]. As discussed above, ORAI channels have been linked to a variety of pathways relevant to growth factor and hypoxia mediated changes in cancer cells. However, there has been limited study of other tumour microenvironment players such as cancer associated fibroblasts and adipocytes (Figure 2). The identification of the important role of Ca^{2+} signalling in the interaction with cancer associated fibroblasts and colon cancer cells[77], and adipocytes with ovarian cancer cells at the metastatic niche[78], highlights the need for the field to define the role ORAI Ca^{2+} channel in these types of tumour microenvironment interactions.

Conclusion

This review has highlighted the increasing number of studies related to ORAI Ca^{2+} channels and cancer. Since the first studies of ORAI1 Ca^{2+} channels in cancer cells, work has progressed to the other ORAI isoforms. One of the major achievements in the field has been a clear demonstration of how changes in ORAI1 and its related isoforms can be very different between different cancer types and even subtypes. However, the field should increase its use of publicly available expression databases to further define cancer subtype specific changes, it must also move towards a clearer and more consistent assessment of expression levels of ORAI and STIM proteins in cancerous versus normal tissue. A variety of studies have defined specific roles for ORAI channels in cancer cell proliferation, invasiveness and cell death and work has started to consider the role of ORAI channels in resistance pathways and key events in the tumour microenvironment. This expansion of our understanding of the role of ORAI channels in cancer progression will no doubt continue, as will our appreciation of new roles for ORAI channels and/or SOCE pathways, such as the recent identification of the role of SOCE loss in radiation therapy induced suppression of salivary gland function via caspase-3 mediated cleavage of STIM1[79]. The next steps will also involve trying to define how this new information regarding ORAI channels in cancer progression and treatment can be best applied to improve patient outcomes. However, the field must also consider the potential side effects of ORAI channel inhibition in particular potential effects on immune pathways important in cancer. The future must also include the use of more diverse and relevant pre-clinical cancer models and a greater utilization of better pharmacological modulators of ORAI1 channels, as well as ORAI2 and ORAI3 selective modulators if they become available.

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Conflict of Interest

G.R.M is associated with QUE Oncology Inc and has patents related to ORAI1 in breast cancer.

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Figure 1: Some selected examples of ORAI and STIM contributions to tumour cell progression. Invasion A. Extracellular matrix degradation (ECM) and cell invasion is orchestrated by oscillations in intracellular calcium concentrations mediated by ORAI1 and STIM1 in the melanoma cell line WM793. Recycling of plasma membrane bound MT1-MMP is dependent on non-constituent calcium entry for successful exocytosis of endocytic compartments[53]. **Proliferation.** Store operated calcium entry drives proliferation in cancer cells in a context specific manner. **B.** Calcium influx mediated by STIM1 in melanoma cell lines C8161, SK-Mel-2 and SK-Mel-24 regulates proliferation through activation of the CaMKII/MEK/ERK pathway[39]. **C.** Activation of heterometric ORAI1/3 channels by arachadonic acid (AA) in LNCaP prostate cancer cells has been demonstrated to drive Ca^{2+} store independent cell proliferation through enhanced activity of transcription factor NFAT[31]. **Migration D.** Decreased SOCE as a result ORAI2 or ORAI1 silencing in the HL60 promyeloblastic cell line reduces phosphorylation of focal adhesion kinase (FAK) and cell migration[29]. Focal adhesion turnover in MDA-MB-231 breast cancer cells is similarly reduced by ORAI1 silencing or pharmacological inhibition through impaired activity of GTPases RAC and RHO[41]. **Resistance to apoptotic signalling and chemotherapeutics E.** Induced overexpression of ORAI3 in T47D breast cancer cells results in reduced levels of pro-apoptotic molecule p53 due to increased ubiquitination by Nedd4-2 and Mdm2. Loss of p53 signalling results in resistance to apoptotic inducers staurosporine and thapsigargin as well as cytotoxic agents cisplatin, 5-fluorouricil and paclitaxel[66].

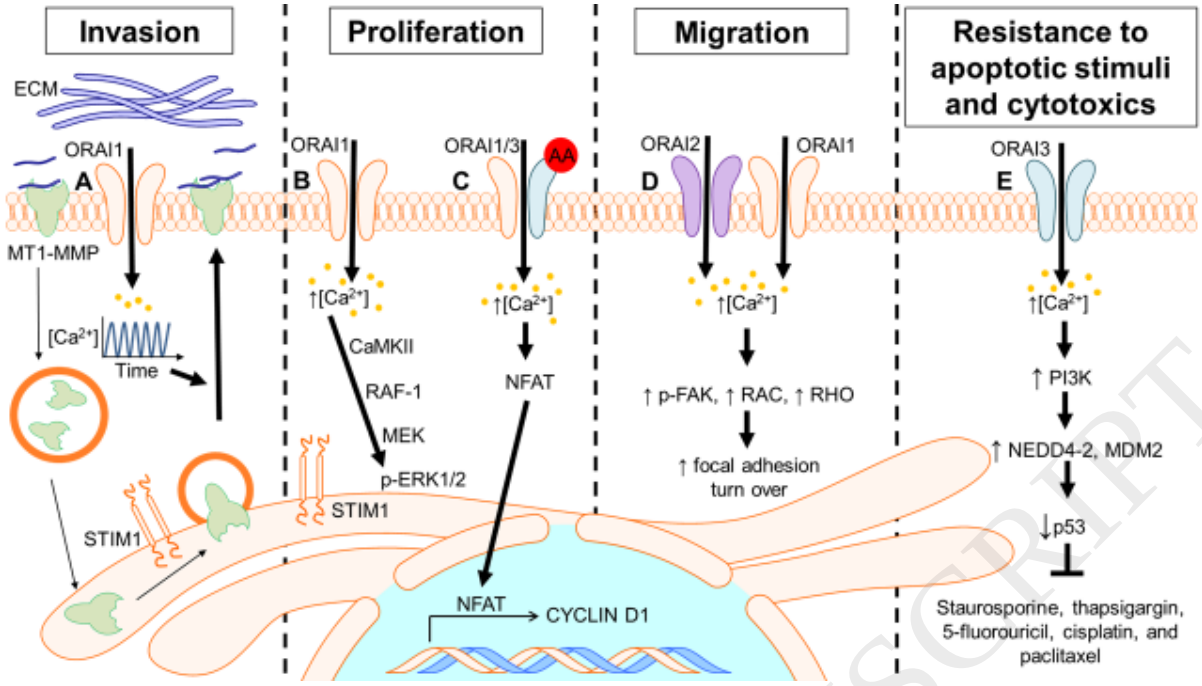

Figure 2. Store operated calcium entry in cells relevant to the tumour microenvironment. Angiogenesis has been linked to ORAI1 or STIM1 dependent Ca^{2+} influx pathways[32, 40, 85], as has immune pathways[71, 72]. Environmental factors including growth factors, extracellular matrix proteins and hypoxia are likely regulators of ORAI and STIM expression and SOCE in a variety of cancer types[32-34, 50, 76]. Although the presence of cancer associated fibroblasts and adipocytes are known to contribute to cancer progression via calcium signalling[77, 78], the role of ORAI and STIM isoforms in these microenvironmental components has not yet been fully elucidated.

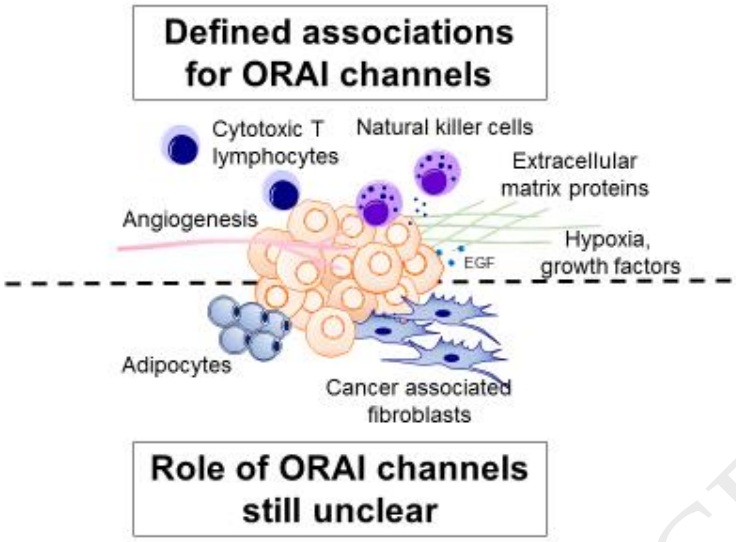

Table 1: Examples of altered expression of ORAI channels and STIM isoforms determined in human cancer clinical samples with matched non-tumourous controls

ORAI

		Change with cancer			
ORAI/STIM isoform	**Cancer type**	**mRNA**	**Protein**	**Change with increasing tumour stage**	**Reference**
ORAI1	Liver cancer	↑	↑		[23]
	Oesophageal cancer	↑	↑	↑	[24]
	Renal cancer		↑		[26]
	Stomach cancer		↑		[25]
	Lung cancer	↑	↑	↑	[80]
ORAI3	Renal cancer		↔		[26]
	Breast cancer	↑	↑		[22, 66]
	Lung cancer	↑	↑	↑	[27, 28]
STIM1	Cervical cancer		↑		[40]
	Stomach cancer		↑	↔	[81]
	Liver cancer	↑	↑		[35, 82]
	Colorectal cancer	↑	↑		[83]
STIM2	Colorectal cancer	↑			[84]