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Calcium channels and cancer stem cells

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ABSTRACT

Cancer stem cells (CSC's) have emerged as a key area of investigation due to associations with cancer development and treatment resistance, related to their ability to remain quiescent, self-renew and terminally differentiate. Targeting CSC's in addition to the tumour bulk could ensure complete removal of the cancer, lessening the risk of relapse and improving patient survival. Understanding the mechanisms supporting the functions of CSC's is essential to highlight targets for the development of therapeutic strategies. Changes in intracellular calcium through calcium channel activity is fundamental for integral cellular processes such as proliferation, migration, differentiation and survival in a range of cell types, under both normal and pathological conditions. Here in we highlight how calcium channels represent a key mechanism involved in CSC function. It is clear that expression and or function of a number of channels involved in calcium entry and intracellular store release are altered in CSC's. Correlating with aberrant proliferation, self-renewal and differentiation, which in turn promoted cancer progression and treatment resistance. Research outlined has demonstrated that targeting altered calcium channels in CSC populations can reduce their stem properties and induce terminal differentiation, sensitising them to existing cancer treatments. Overall this highlights calcium channels as emerging novel targets for CSC therapies.

1. Introduction

Cancer incidence and mortality has been rising steadily with global cancer data estimating that in 2018 there will be 18.1 million new cancer cases and 9.6 million deaths, making it the leading cause of death worldwide [1]. Despite treatment advances resulting in better patient outcomes there is still more that needs to be done to improve long term survival. Cancers are most commonly treated with a combination of surgery, chemotherapy, and radiotherapy all of which have proven efficacy, however research has demonstrated that they fail to target or actively select for resistant cell populations termed cancer stem cells (CSC's) [2]. Emerging hypotheses suggest that CSC's are the cell of origin promoting, tumour development through their ability to self-renew and differentiate into a multi-lineage of cells [3–8]. Furthermore, they can also remain quiescent and resist apoptosis which has resulted in a link to treatment resistance and tumour relapse [9–11]. A number of studies have demonstrated the presence of CSC's across various cancers and confirmed a role in treatment resistance and poorer patient prognosis [5,7,11,12]. Improving our understanding of CSC's and the underpinning mechanisms that drive their functions could uncover new cancer treatment targets. Calcium is fundamental for normal cellular functions such as proliferation, survival, differentiation and gene expression in a range of cell types including stem cells [13,14]. Alterations to calcium channels that govern intracellular calcium has been linked to the development of a number of pathologies including cancer [15–17]. More recently research has highlighted that ion channels, including calcium channels, play a role in CSC function [18]. This review will explore in detail the current state of the art pertaining to our knowledge on CSC's and how calcium channels form part of an integral mechanism required for their function, enabling cancer progression and treatment resistance.

Abbreviations: AML, acute myeloid leukaemia; ALDH1, aldehyde dehydrogenase 1; AA, arachidonic acid; CRC, calcium release channel; CSC's, cancer stem cells; GFAP, glial fibrillary acidic protein; GSC's, glioblastoma stem cells; GSTO1, glutathione S-transferase omega 1; HCSC, hepatocellular carcinoma stem cells; IP3R, inositol 1,4,5-triphosphate receptor; NSLC, non-small cell lung cancer; OSCC, oral/oropharyngeal squamous cell carcinoma; PM, plasma membrane; PIP2, phosphatidylinositol 4,5-bisphosphate; RYR, ryanodine receptor; SICE, store independent calcium entry; SOCE, store operated calcium entry; STIM, stromal interaction molecule; TRPC, transient receptor potential cation; TRP, transient receptor potential; VGCC, voltage gated calcium channels

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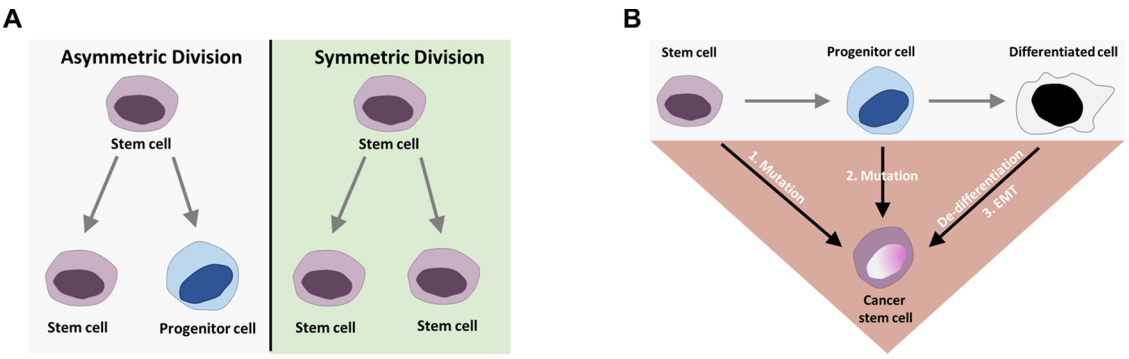

Fig. 1. Cancer Stem Cell Division and Origin. (A) Stem cells can undergo symmetric division generating two identical daughter cells replenishing the stem cell pool or asymmetric division producing a stem and progenitor cell, the later differentiating with specialised function. In cancer, symmetric division of CSC's is enhanced increasing overall numbers, followed by aberrant asymmetric division to generate tumour bulk. **(B)** Stem cells are able to differentiate into a full lineage of cells (Grey shaded area). Mutations, EMT or influence by the niche environment contributes to plasticity of this hierarchy and promotes the development of cancer stem cells (CSC's).

2. Cancer stem cells (CSC's)

Stem cells give rise to a variety of differentiated cell lineages and thus play a key role in the maintenance and regeneration of tissue in healthy individuals [4]. The population of stem cells avoid depletion due to their unique capacity of self-renewal, achieved through two types of cell division (Fig. 1A). Symmetric division is where two daughter stem cells are generated, identical to the parent stem cell ensuring maintenance of the stem cell pool. Asymmetric division results in the production of a stem cell and progenitor cell, the latter of which differentiates into a terminal cell with specialised function, demonstrating their multi-lineage formation capacity [19,20]. The type of division that occurs is governed by a number of factors such as the surrounding cells, environmental niche and acquired mutations [21]. It is thought that all of these are impacted in cancer and as a consequence promotes the transformation of these cells into cancer stem cells (CSC's), driving their abnormal division (Fig. 1B) [5,22]. Research attributes the resultant tumour microenvironment as a key factor that enhances symmetric division leading to the expansion of CSC populations, which ultimately undergo aberrant terminal differentiation generating the heterogeneous tumour bulk [5,19,22]. Current treatments generally exert their effects on differentiated proliferating cells, but CSC's are resistant as they exist in a dormant quiescent state [7,9]. While differentiated cells have limited replicative potential, the CSC's ability to self-renew allows it to repopulate the tumour bulk following treatment promoting recurrence (Fig. 2).

Although CSC's display self-renewal and differentiation characteristics like "normal" stem cells their origin is still unclear, however several mechanisms have been hypothesised (Fig. 1B) [5,12,23]. It has been proposed that the pathways regulating normal stem cells may become altered due to mutation, promoting self-renewal and uncontrolled proliferation. Stem cells have a longer lifespan than differentiated cells making them more likely to experience mutagen exposure [19]. On the other hand, progenitor cells or terminally differentiated cells could also revert to a stem like phenotype following oncogenic mutation reactivating self-renewal genes previously lost upon differentiation [24]. Alternatively, cells may undergo epithelial-to-mesenchymal transition resulting in a stem like phenotype [25–27]. Originally it was presumed that given the typical cell hierarchy that a mutated stem cell was the likely candidate. However more recent research has disputed this notion highlighting that cell hierarchies display significant plasticity and that at any stage a cell is able to undergo reprogramming to become a CSC. Emerging studies have attributed this to altered signals generated from the tumour microenvironment (Fig. 1B) [5,12,21].

Taken together this supports the hypothesis that CSC's are involved not only in tumour development but also treatment resistance and recurrence (Fig. 2), highlighting them as an attractive novel target in cancer treatment [12]. Despite this our lack of knowledge into the mechanisms that drive CSC function along with heterogeneity, make identifying and eradiating CSC's more difficult than originally thought [5,12]. However, this need not comprise future therapy potential but requires a further understanding into common mechanisms across various CSC's. Here in we explore how changes in intracellular calcium regulated by calcium channels represents one such potential targetable common mechanism involved in CSC function.

3. Calcium channels and cancer

Calcium is fundamental for cellular activity under both normal and pathological conditions, with research highlighting its ability to regulate a number of key functions such as proliferation, contraction, secretion, migration, survival, apoptosis and gene expression [28]. Ultimately changes in intracellular calcium are initiated and controlled by calcium channels through two potential mechanisms [29]. The first is via calcium influx through calcium permeable plasma membrane proteins such as the voltage gated calcium channels (VGCC), store operated calcium entry (SOCE) channel ORAI, and some transient receptor potential channels (TRP) [29]. The second is calcium release from intracellular stores such as the endoplasmic reticulum (ER) through calcium release channels such as inositol 1,4,5-triphosphate receptors (IP3R) or ryanodine receptors (RYR). IP3R channels are opened

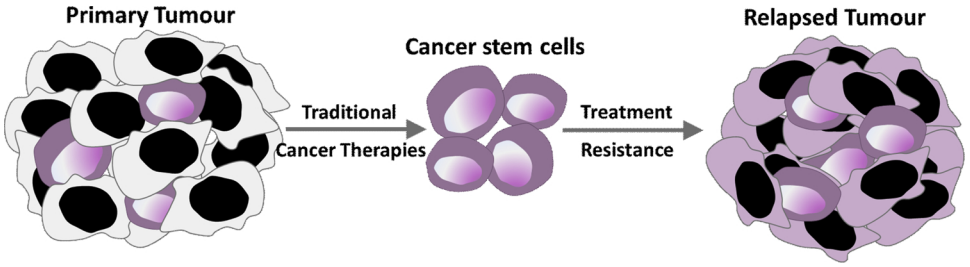

Fig. 2. Treatment resistance and tumour relapse promoted by cancer stem cells. Current cancer treatments target the bulk of actively proliferating cancer cells but quiescent CSC's are resistant to these treatments. Remaining CSC's promote tumour redevelopment. These relapsed tumour cells often carry characteristic traits of the resistant CSC's that gave rise to them.

Table 1
An overview of the currently known calcium channels or associated subunits that are linked to a cancer stem cells and their assocaited functional role.

Channel	Cancer Type	Function	Ref
4.1 VGCC			
T-type CaV3.2	Glioblastoma	Enhances CD133 stem populations. Inhibition reduced stem cell properties and sensitised stem cell populations to chemotherapy treatment.	[60]
L-type CaV1.2, CaV1.4 N-Type CAV2.2	Hepatocellular carcinoma	Increased α2δ1 subunit induced the expression of VGCC resulting in increased basal calcium levels, promoting increased self-renewal and tumour formation.	[65]
L-Type α2δ1 subunit	Non-small cell lung cancer	High expression associated with self-renewal, tumour formation and radio and chemotherapy resistance – reversed by knockdown.	[66] [67]
4.2 CRC			
RYR1	Breast cancer	Knockdown of RYR1 reduce stem cell populations and associated pathway Nanog.	[74]
RYR3	Medulloblastoma	Downregulation of RYR3 enhanced Nanog and CD133 expression and promoted spheroid formation.	[75]
IP_3R	Melanoma	Decrease IP_3R mediated calcium flux reduced expression of stem pathways.	[76]
4.3 SOCE			
ORAI1	Oral/oropharyngeal squamous cell carcinoma (OSCC)	ORAI1 expression high in stem cell populations. Linked to self-renewal, migration, and transcription factors such as Nanog and OCT3/4. Promoted stemness through activation of NFAT pathway.	[78]
4.4 TRP channels			
TRPv2	Oesophageal Liver Glioblastoma	ALDH positive stem cells presented increased TRPV2 expression. Inhibition reduce stem markers and self-renewal. Liver cancer stem cells had decreased TRPV2 expression. Overexpression reduced stem cell markers and self-renewal capacity. Overexpression of TRPV2 induced stem cell differentiation and reduce self-renewal capacity. Induced TRPv2 expression using cannabidiol enhanced existing chemotherapy treatment, inducing apoptotic death of CSC's.	[85] [86] [87] [88]
TRPM7	Glioblastoma Neuroblastoma	TRPM7 expression is high in stem cell populations along with STAT3 and survivin. Required for stem cell self-renewal and differentiation. TRMP7 is linked to development and differentiation pathways. Promoting the expression of genes linked to stemness such as STAT3, WNT1 and NOTCH1.	[89] [90]

following G protein coupled receptor or receptor tyrosine kinase activation, while flux through RYR's is triggered by the presence of calcium as a result of membrane depolarisation activating VGCC's [30]. SOCE is triggered to refill intracellular stores via store operated channels, consisting of the calcium sensing S/ER stromal interaction molecule (STIM) and pore forming component, ORAI [31]. Upon store depletion STIM dimers move to the ER-plasma membrane (PM) junction, where following a conformational change they interact with and trigger the activation of ORAI channels promoting calcium influx [32]. Alternatively, STIM can also promote calcium entry through the Transient Receptor Potential Cation (TRPC) Channels [33].

Furthermore, changes in intracellular calcium mediated by calcium channels are also known to activate a number of calcium dependent downstream effectors to control cellular processes, under both normal and oncogenic conditions [17,34,35]. For example, Calmodulin (CaM), regulates cell cycle and proliferation through its function on targets such as Ca2+/calmodulin kinases (CaMK), calcineurin (CaN), ERK, NF-κB and the cAMP response element (CREB) [34]. Calcineurin is also known to activate nuclear factor of activated T cells (NFAT), originally associated with promoting T-cell proliferation but more recently linked to proliferation, migration and resistance across various cancer types [36]. Activation of NFAT and also ERK pathways has been shown to be modulated by calcium influx through TRP channels as well as other calcium channels in cancer cells [17,34,35]. Not surprisingly research has highlighted a role for these effectors in stem and progenitor cell functions [37–40] and their disruption in CSC's [41].

The spatiotemporal nature of transient fluctuations in intracellular calcium is decoded by the cell, allowing it to control a wide variety of cellular functions, including those contrasting such as proliferation and apoptosis [15]. For example, small increases in intracellular calcium promotes proliferation, whereas apoptosis is triggered following sustained release. As a result calcium remodelling during oncogenic transformation can confer an advantage, highlighted by extensive research linking such changes to cancer development and progression [17,35,42–45]. Accordingly, it has been demonstrated that increases in calcium channel expression could promote cancer cell proliferation and or migration, whereas decreases in expression can promote apoptotic resistance. As such the expression of calcium channels has been demonstrated to be both upregulated and downregulated in various different cancer types and both shown to be associated with a survival benefit [46,47]. Owing to the fact that calcium disruption is associated with oncogenic transformation, it has been highlighted as a promising target for personalised anti-cancer therapies [48].

4. Calcium channels and cancer stem cells

Recent research has shown that changes in intracellular calcium are also important in the self-renewal capacity, proliferation and differentiation of stem cells, with a wide variety of calcium channels such as VGCC and store release channels, IP3R and RYR implicated [13,14,49]. The expression of a number of VGCC's has been confirmed across a range of stem cell types and differentiation states but their physiological role still needs to be identified [13,14,49]. T-type VGCC currents have been linked to cell cycle progression and maintenance of self-renewal capacity in undifferentiated stem cells [50]. Whereas L-type VGCC's seem to be linked to stem cell differentiation [49,51]. In terms of calcium store release, IP3R channels appear conserved across all stem cell types and contribute significantly to calcium oscillations particularly in undifferentiated stem cells, controlling proliferation and differentiation [49,52–54]. Information regarding the expression and function of RYR channels is limited but initial work links them to stem cell differentiation [49]. Similar to the observations in cancer, it is suspected in CSC's that changes in intracellular calcium due to altered calcium channel expression could also confer an advantage in their ability to support cancer progression. As a result these channels could also represent important targets against CSC's. Taking this into account it is natural to hypothesise that aberrant calcium oscillations might contribute to altered CSC function promoting cancer development and resistance. Below we explore this hypothesis by outlining a comprehensive overview of calcium channels linked to CSC function (Table 1 and Fig. 3A).

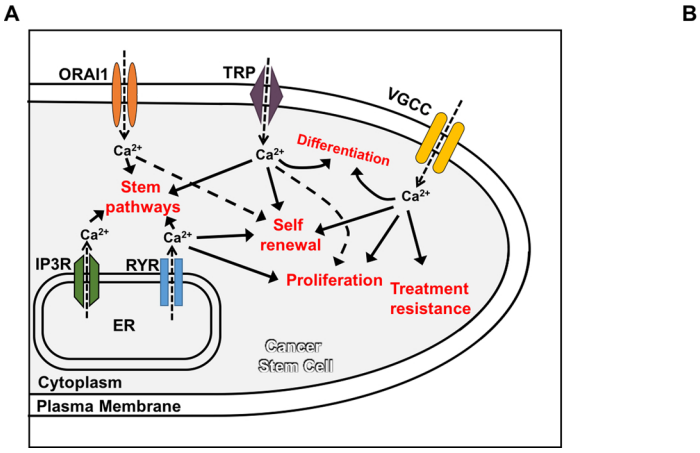

Fig. 3. Cancer Stem cell treatment targets. **(A)** There are a number of calcium channel families such as VGCC, TRP as well as store operated ORAI, RYR and IP3R that alter intracellular calcium levels in cancer stem cells. Each of these channels can contribute to a number of stem properties such as the activation of stem pathways, self-renewal, treatment resistance and differentiation. **(B)** Targeting these calcium channels on CSC's could provide a therapeutic benefit. Manipulation of which could induce apoptosis of CSC's directly or induce terminal differentiation allowing eradiation with existing treatments.

4.1. Voltage gated calcium channels (VGCC)

Voltage gated calcium channels (VGCC) are a family of ion channels found on the plasma membrane activated following its depolarisation. There are a number of sub-types each with varying biophysical properties, for example T-type VGCC's, are known as 'transient' displaying fast kinetics of activation and inactivation with a low activation threshold of −60 mV, peaking at −20 mV [17,45,55]. Alternatively, L-type channels are known as 'long-lasting' and are characterised by a large single channel conductance amplitude with a high activation threshold of −40 mV peaking at 0−20 mV depending on the cell type, channel variant and splice form [45,55]. Research has shown that the membrane potential of cancer cells is more depolarised compared to their normal counterparts with a resting membrane potential around −30 mV to 0 mV [56]. Furthermore, a similar depolarised resting membrane potential has also been witnessed in both normal and cancer stem cells [57–59]. As a result, it is likely that cancer and CSC's display the correct voltage window for VGCC's to be active if expressed.

The only T-type VGCC found to be present on CSC's thus far is, CaV3.2, where its upregulation was observed in glioblastoma stem cell (GSC) populations and linked to a role in promoting proliferation [60]. Treatment of GSC's with CaV3.2 inhibitor, mibefradil, inhibited their proliferation and induced cell death. Furthermore, it also promoted stem cell differentiation as evidenced by the downregulation of stem related genes such as Nestin, CD133 and Sox2 and the upregulation of those associated with astrocyte differentiation such as Glial fibrillary acidic protein (GFAP). As suggested here in, there is potential benefit to targeting CSC's to enhance existing treatments and reduce resistance. This study clearly supports this by demonstrating that targeting GCS in a xenograft model with mibefradil alongside current chemotherapy treatment, temozolomide (TMZ), significantly reduced tumour size and prolonged survival compared to chemotherapy alone. The role of CaV3.2 in stem cell function appears conserved across stem cells as work with mouse embryonic stem cells showed that knockdown or blockage of the channel with Ni^{2+} reduce stem cell proliferation and gene expression of stem cell pathways OCT3/4 and Nanog, indicating a loss in self-renewal capacity [61]. Together this work suggests that calcium mobilisation through CaV3.2 plays a key role in the stem cell proliferation and differentiation state.

VGCC's transport calcium through a large alpha pore, however there are a number of associated accessory subunits that can fine tune its properties [62,63]. One such subunit, alpha 2 delta 1 (α2δ1), has been shown to control channel trafficking, localisation and biophysical properties, thus having significant influence over the channels calcium influx [64]. Research has demonstrated that the α2δ1 subunit is highly expressed in CSC's and relates to their function [65–67]. One study found that expression of α2δ1 was greater in hepatocellular carcinoma stem cells (HCSC) compared to non-stem like controls and that its knockdown lead to reduced self-renewal and tumour formation capacity as well as apoptotic induction [65]. Due to the link between the subunit and the calcium conducting alpha pore of VGCC's its ability to influence calcium mobilisation and how this affects stem cell function was investigated. It was noted that overexpression of α2δ1 in HCSC's significantly increased basal calcium levels, whereas knockdown or inhibition with a monoclonal antibody reduced basal calcium and spontaneous calcium oscillations. Chelating extracellular calcium with EGTA also reduced spontaneous calcium oscillations suggesting the α2δ1 was controlling influx of extracellular calcium through plasma membrane channels such as VGCC's rather than intracellular stores. Furthermore, α2δ1 overexpression significantly increased expression of a number of VGCC's including, L-type CaV1.2 and CaV1.4, as well as N-type CaV2. Together this suggests that α2δ1 regulates intracellular calcium by controlling extracellular calcium influx through the subunits association with L or N-type VGCC's, a mechanism which has been previously described in other cell types [64]. This was confirmed using L, N and T-type channel inhibitors which significantly reduced self-renewal capacity in a range of HCSC's confirming a link between α2δ1 and calcium mobilisation through VGCC. Furthermore, the study also noted that treatments of hepatocellular carcinoma mouse models with a α2δ1 targeting antibody reduced treatment resistance associated with chemotherapy alone. In doing so they noted a significant decrease in tumour weight and size as well as prolonged survival with combination treatment, supporting the notion that targeting CSC's could overcome treatment resistance and improve patient survival.

Two subsequent studies in non-small cell lung cancer (NSLC) stem cells confirmed this link between high α2δ1 expression with enhanced self-renewal capacity and treatment resistance [66,67]. Both studies demonstrated that the subunit was upregulated in NSLC stem cell populations and that its expression correlated with enhanced self-renewal capability, as evidenced by increased tumour spheroid numbers, as well as an associated increase in stem pathways Nanog and OCT4 and stem cell marker CD133 [66,67]. In addition, they also highlighted that targeting the subunit with an antibody overcame radio and chemotherapy resistance, further reducing tumour size compared to individual treatments [66,67]. Unlike the aforementioned work on HCSC these two subsequent studies failed to confirm a direct association between the subunit and calcium influx as a potential mechanism in CSC function. Considering the links between α2δ1 and VGCC function in a range of cells it is likely that a similar mechanism drives the observations witnessed, however further work is needed to confirm this hypothesis and that α2δ1 is not acting in a non-canonical manner [64].

4.2. Calcium release channels (CRC)

4.2.1. Ryanodine receptors (RYR)

Ryanodine receptors (RyR's) are important in regulating calcium

store release from the endoplasmic reticulum (ER) in a range of cells, and while primarily associated with muscle contraction, research has started to uncover links to other cellular functions [30,68]. Studies have demonstrated their altered expression and function in cancer, linking them to roles in proliferation and apoptosis as well as correlating expression with tumour grade [69–72]. Tentative links to normal stem cell function have started to emerge indicating a role in differentiation and more recently associations to CSC's have been noted [49]. For example in breast cancer (BC), chemotherapy was shown to lead to CSC enrichment through the induction of the glutathione S-transferase omega 1 (GSTO1) biosynthesis pathway [73]. Subsequent research by the same group highlighted that RYR1 formed part of this mechanism promoting breast cancer stem cell (BCSC) enrichment [74]. It was observed that RYR1 expression was higher in chemotherapy resistant BCSC's, within which the GSTO1 pathway promoted calcium release from the ER through the channel. Channel knockdown resulted in a reduction of stem cell populations and expression of the stem pathway, Nanog. Alternatively, RYR1 activation enhanced these populations, indicating that calcium store release is required for BCSC proliferation. Using BC mouse models they found that targeting RYR in combination with chemotherapy treatment resulted in a significant reduction in tumour volume, suggesting that targeting CSC's alongside existing treatments could enhance outcomes [74]. In another study, down regulation of RYR was associated with promoting a stem like phenotype in medulloblastoma [75]. It was noted that the miRNA, miR-367, was upregulated leading to decreased RYR3 expression [75]. This enhanced the formation of spheroid cultures indicating increased self-renewal, along with an associated increase in the expression of stem pathways and markers, Oct4 and CD133 respectively [75]. However, RYR3 was downregulated alongside a number of other genes, thus additional work is required to confirm a direct association between the channel and its ability to promote a stem phenotype. Overall, these investigations provide evidence linking RYR's to CSC's and their function but does show that differing isoforms and expression patterns can have contrasting effects on stem cell properties. As such further research is required in this area before these channels could become viable targets.

4.2.2. Inositol trisphosphate receptor (IP_3R)

As noted, IP_3R's mediate the release of calcium from the endoplasmic reticulum following stimulation by IP_3, disruption of which has been directly linked to oncogenesis in a number of different cancer types [48]. Furthermore, these channels appear conserved across various stem cell types, with research demonstrating that they play a role in proliferation and differentiation [49,52–54]. Currently, only one study has indirectly linked IP3R's to promoting a CSC phenotype. Marciel et al had previously shown that Selenoprotein K (SELENOK) was required to trigger calcium flux through IP_3R and given the link of IP_3R to oncogenesis they wanted to determine if a similar mechanism could play a role in melanoma [76]. Using CRISPR/Cas9 to generate SELENOK deficient melanoma cells they found that IP_3R function was impaired leading to reduced intracellular calcium, resulting in reduced cell proliferation, migration and invasion. Interestingly gene array analyses showed that cells with impaired IP_3R function due to SELENOK knockdown also had a significant reduction in genes associated with stemness such as PROM1. They concluded that SELENOK expression is required for IP_3R dependent maintenance of stemness [76].

4.3. Store operated calcium entry channels (SOCE)

ORAI, as outlined, is involved in replenishing intracellular Ca^{2+} stores following their depletion and its dysregulation has been consistently linked to cancer pathogenesis [44,48,77]. More recently a role for ORAI1 in CSC's, namely oral/oropharyngeal squamous cell carcinoma (OSCC) stem cells has been established [78]. This study witnessed that ORAI1 expression increased throughout disease progression and that it was also upregulated in CSC populations of OSCC. This observation is interesting considering CSC's are thought to generate tumour bulk thus it would be expected that the terminally differentiated cancer cells would have phenotypic traits associated with the CSC's that they were born out of. The importance of ORAI1 in promoting stem like traits was confirmed when its overexpression in non-tumourigenic oral epithelial cells triggered the development of populations with enhanced stem cell properties such as self-renewal and the upregulation of stem cell transcription factors and markers. This role being further confirmed when channel knockdown reversed these associated CSC traits. In addition, it was highlighted that the NFAT signalling pathway formed part of the mechanism through which ORAI1 promoted these stem cell properties. NFATc3 was found to be upregulated and localised to the nucleus following ORAI1 overexpression and that its knockdown was able to reduce self-renewal to similar levels witnessed following ORAI1 knockdown, suggesting the two are interlinked in OSCC stem cell function. More work however is needed to investigate ORAI channels in CSC's, as well as any associated pathways.

4.4. TRP channels

Transient receptor potential (TRP) channels are a family of approximately 30 membrane proteins that are involved in the transport of cations such as Ca^{+}, Mg^{2+} and Na^{+}, responding to a wide range of chemical and physical stimuli often associated with sensing [79]. Owing to their ability to mobilise calcium they have thus been implicated in a variety of cellular processes such as cell cycle, proliferation, apoptosis and migration [80]. Changes in their expression or activity has been connected with several cancer hallmarks across a number of different cancer types, as reviewed in detail here [81]. To date only a few of the family members have been linked with stem cell function and with regards to CSC's only TRPV2 and TRPM7 have been associated [82].

Early studies have shown that TRPv2 is expressed in normal stem cells but have not determined what impact it has on their function [83,84]. However, more recently TRPv2 has been directly linked to self-renewal of CSC's in a number of cancer types such as oesophageal [85], liver [86]and glioblastoma [87]. A study by Shiozaki et al conducted a microarray analysis on cancer stem cells isolated from oesophageal squamous cell carcinoma by selecting for cells expressing high levels of the stem cell marker Aldehyde dehydrogenase 1 (ALDH1) [85]. It was observed that these CSC's had significantly altered expression of a number of ion channel families such as potassium, calcium, chloride and TRP. Confirmation of the bioinformatic analysis using PCR highlighted TRPv2 as a possible candidate that was significantly upregulated in their CSC population compared to control. Further investigation found that treatment of CSC's with the TRPv2 inhibitor, tranilast, resulted in a significant reduction of CSC populations and self-renewal capability. Implying that targeting CSC's with tranilast could represent a potential treatment to remove CSC's and reduce the risk of relapse.

However, studies of TRPv2 in CSC's of other cancer types observed a reverse correlation, where decreased channel function or expression enhancing stem cell traits. One such study established that TRPv2 was decreased in liver CSC populations compared to normal tissue. Liver CSC populations demonstrated an increase in stem cell markers such as CD44, CD133 and ALDH1, which correlated with a decrease in TRPv2 [86]. Knockdown of the channel further enhanced stem cell populations by increasing self-renewal capacity, while overexpression reversed this and also decreased stem cell markers. Xenograft liver cancer mouse models correlated with cell line models where decreased expression of TRPv2 enhanced tumour growth and overexpression had the opposite effect. In agreement to previous observations, Morelli et al, demonstrated that TRPv2 expression was lower in glioblastoma stem cells compared to their differentiated counter parts [87]. Differentiation of stem cells resulted in a stepwise increase of TRPv2 expression which correlated with upregulated basal calcium levels as well as an increase in differentiation marker GFAP and a reduction in stem pathway,

nestin. This correlation was confirmed using channel manipulation with both channel inhibition and knockdown showing a decrease in terminal differentiation marker, GFAP, whereas overexpression induced GFAP and reduced self-renew capacity. To ascertain if TRPv2 activity was the mechanism involved, differentiated cells were treated with channel activator, cannabinoid, cannabidiol (CBD) and an increase in basal calcium observed. Similar findings also emerged when translated to xenograft mouse models, with TRPv2 overexpression reducing tumour diameter and promoted differentiation of stem cells to a terminal tumour cell. Later work by another group witnessed similar observations when using CBD to stimulate the expression of TRPv2 in glioblastoma stem cells [88]. They found that TRPv2 expression was increased by CBD treatment and that this resulted in reduced stem cell proliferation and self-renewal capacity correlating with an increase in terminal differentiation marker, GFAP and decrease in stem cell markers CD133 and Oct4. While this was initiated by the induction of TRPv2 expression following CBD treatment, the effects were prevented by the TRPv2 inhibitor ruthenium red, suggesting that they were mediated through the canonical function of this channel. Considering the ability of CBD to reduce stem cell populations the potential of this compound in treating glioblastoma was tested. Combination treatment with traditional chemotherapy agent, Carmustine, was able to induce apoptosis of GSC's and improved outcomes on tumour progression. Taken together this work shows that TRPv2 does play a key role in the proliferation, self-renewal and differentiation capacity of CSC's and that compound's which manipulate channel function or expression could be used as a potential CSC therapeutics. However, the different roles this channel plays across different CSC's types suggest that further research is required to ensure the correct compounds are used in targeting specific cancer types.

TRPM7 is another TRP family member, which is uniquely known to not only transport calcium and magnesium but also act as a serine/threonine kinase [89]. Its aberrant expression has been to linked to proliferation and metastatic potential across numerous cancer types [81]. Two studies to date have also demonstrated TRPM7 expression in CSC's, namely glioblastoma and neuroblastoma, where it has been associated with promoting stem traits and metastatic potential [89,90]. Lui et al highlighted that glioblastoma stem cells had high levels of TRPM7 expression compared to differentiated controls [89]. Knockdown significantly reduced stem cell markers CD133 and ALDH1 as well as stem pathways STAT3 and Notch, suggesting that it is involved in regulating differentiation and self-renewal [89]. Lastly, a study on neuroblastoma cells observed that increased levels of TRMP7 were linked to enhanced metastatic potential and wanted to investigate if this was related to previous work linking the channel to embryogenesis and maintenance of progenitor cells [90]. To identify any associations shRNA was used to knock down TRPM7 in the neuroblastic cell line, SH-SY5Y, and a microarray and bioinformatic analysis conducted. They discovered that TRPM7 was linked to cell development and differentiation pathways, specifically genes associated with stemness such as STAT3, WNT1, Notch1 and SNAI1. Overall, they suggest that TRPM7 is linked to enhancing a stem cell phenotype in neuroblastoma which could be involved in promoting tumour development and metastatic spread. Despite this, more work is needed to confirm a direct association between TRPM7 expression and stem cell traits. Both of these studies failed to differentiate if the effects mediated by TRPM7 were due to kinase activation or channel activity. However further work subsequently published by both groups using the same cell lines as in their retrospective studies demonstrated that the TRPM7 channel was active and regulated calcium influx [91,92]. While this suggests that associated stem cell traits were mediated through the channels canonical function further work is needed to confirm such a hypothesis.

5. Conclusion and future directions

CSC's are known to play a key role in cancer development and treatment resistance. Current treatments target the tumour bulk but either miss or enhance CSC populations which subsequently promote treatment resistance and tumour relapse (Fig. 2). Targeting CSC's is thought to represent a potentially novel therapeutic strategy that could ensure complete tumour removal reducing the risk of recurrence. Despite this potential, issues around CSC heterogeneity along with recently highlighted stem cell hierarchy plasticity have created challenges, requiring new treatments that target mechanisms and or pathways common across a heterogeneous CSC population [12]. Here in we have outlined how calcium mobilisation through calcium channels drives CSC function and could represent a new approach to targeting CSC's (Fig. 3A). We found that a wide variety of different classes of calcium channels were aberrantly expressed in CSC's and linked to stem cell characteristics (Table 1). Indeed, studies demonstrated that channel manipulation reduced CSC populations by decreasing proliferation, inducing apoptosis and or differentiation. Furthermore, targeting these CSC's alongside existing treatments enhanced their effects and improved outcomes further highlighting the therapeutic benefit to targeting CSC's (Fig. 3B).

While calcium channels appear to represent potential novel CSC targets further work is needed for the development of successful therapeutics. As highlighted there are many calcium channels that have not been investigated in the context of CSC's. Furthermore, of the channels outlined their expression or function was not consistent across different cancer types. Thus further research is required to ensure that calcium channels targeted are modulated correctly in order to produce the desired effects; otherwise it could potentially enhance CSC populations. In terms of drug development targeting calcium related targets is a relatively new field compared to other drug target areas. This is in part due to the complexity of the calcium signalling network however as our understanding has improved the number of drug targets in this area has increased in recent years with some moving into clinical trials, as highlighted in this review [48]. Due to common reliance on the calcium signal in various normal and pathological tissues, issues around tissue specificity and the ability to explicitly target cancer cells need to be addressed to reduce off target effects [48]. These issues could potentially be circumnavigated through the identification of cancer specific biomarkers and the use of targeted delivery systems [93–95]. However, a benefit of targeting ion channels such as calcium channels is the large body of FDA approved drugs already available to manipulate them [96,97]. This presents significant opportunities to repurpose existing drugs targeting calcium channels on both cancer cells and CSC's for therapeutic benefit [45].

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