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Review

Calcium signaling regulates fundamental processes involved in Neuroblastoma progression

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ABSTRACT

Neuroblastoma (NB) is the most common extra-cranial pediatric solid tumor in children. Despite NB's relative rarity, high-risk NB patients have a poor prognosis with survival rate less than 50%. This is even worse for patients with relapsed or refractory NB. Finding effective alternative treatment strategies is therefore a must.

Calcium is an intracellular messenger that is unequivocally present in normal physiology mediating proliferation, growth, migration, cell division, angiogenesis and cell death, as well as pathophysiological processes such as those included in Weinberg's hallmarks of cancer. Within the past 20 years, the molecular identity of most calcium channels has been revealed, however for some of these channels the precise gating mechanism and their role in normal physiology is still elusive.

Here we review the recent findings of components of calcium signaling that are deregulating in the malignant progression of NB. We further integrate critical calcium signaling pathways using patient-derived expression analysis.

Revealing the roles of these calcium pathways in tumor development, progression, microenvironment and importantly - protection against antineoplastic drugs may hopefully lead to novel treatment strategies in the future.

1. Introduction

Neuroblastoma is a pediatric extracranial tumor that is derived from the neural crest cells of the peripheral sympathetic nervous system. NB is responsible for 15% of pediatric cancer related deaths in the US [1]. Most primary tumors occur in the adrenal glands, and sympathetic ganglia. Poor prognosis is associated with *MYCN* amplification, stage 4 disease, segmental chromosomal mutations, and diploidy [2,3] [4,5]. NB metastasis is commonly observed in bone, bone marrow, lymph nodes, liver or other organs. Using known clinical features and pathobiological markers, NB may be divided into low, intermediate and high risk for relapse. Patients with low and intermediate risk NB have over 95% survival. NB patients with high risk for relapse have less than 50% survival rate. NB tumors are highly heterogeneous, and are comprised of cells with varying molecular features that influence tumor progression and treatment response [6–9].

Children diagnosed at the age of 18 months or older are more likely to have high risk NB and poor prognosis, and approximately 70% of NB patients present with widespread dissemination at the time of diagnosis. In addition, there are somatically acquired chromosomal alterations that are frequently associated with advanced stage, high risk NB and poor outcome. These include *MYCN* gene amplification and segmental chromosomal mutations in 1p, 11q and 17q, as well as other mutations [5,10–12].

The MYCN protein is a basic helix-loop-helix (bHLH) Transcription Factor that is encoded by the *MYCN* proto-oncogene. It is homologous to the v-myc myelocytomatosis viral related oncogene but distinct from the more commonly known c-myc [13–15]. *MYCN* is amplified in a number of cancers, particularly in Neuroblastoma [16,17]. Using fluorescence *in situ* hybridization (FISH) [18,19], the fluorescence associated with *MYCN* gene copy number may be amplified over 10 times, with an average of 100–200 copies/cell, and increasing *MYCN* copy number correlates with unfavorable outcomes. Approximately 16–25% of NB tumors have *MYCN* gene amplification, which is associated with poor prognosis. In high risk NB patients, approximately 40–50% have *MYCN* amplification [20,21]. MYCN regulates the expression of a plethora of genes whose protein products regulate fundamental physiological processes in cells, many of which are dysregulated in NB. These include, but are not limited to, *NME2, CRABP2, LIF, MDM2, MIR17HG, PRMT1, MCM7, MCM8, ODC1, BIRC5, LUC7L, TWIST1, RAB5C,*

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AURKA, H1 F0, MYBL2, and *TP53* [22].

The *TP53* gene encodes the p53 protein, which functions as a tumor suppressor by regulating genes involved in cell cycle arrest and apoptosis [23,24]. It functions as a transcription factor in the nucleus is well documented. However, recent studies have reported that p53 also has a unique function in the cytoplasm. The p53 protein was observed to enhance SERCA activity, increase ER calcium levels, promote calcium signaling between the ER and mitochondria, promote mitochondrial calcium overload and induce apoptosis [25]. The dual roles of p53 has significant influence over the progression of cancers, including NB.

Cell surface receptors TrkA and TrkB are neurotrophin receptor kinases that may play a role in NB. TrkA is present mostly in low risk NB, and the absence of the TrkA ligand neurotrophin growth factor (NGF) is associated with spontaneous regression. This suggests that TrkA signaling may play a role in slowing NB progression. Alternatively, TrkB is expressed in high risk NB, and the expression of its ligand brain derived neurotrophic factor (BDNF) promotes NB progression [26,27]. TrkB plays a role in regulating calcium signaling via BDNF-induced Phospholipase C [28].

Anaplastic lymphoma kinase (ALK) is a receptor Tyrosine Kinase that is expressed at high levels during embryonic development and in neonatal brains [29]. There are over 35 known ALK mutations. Approximately 10% of high-risk NB patients have mutated ALK gene, which predominantly results in gain of function. Germline mutations in ALK may be found in familial NB, while somatic mutations result in oncogenic activation of ALK [30]. The most prevalent ALK mutation involves chromosomal rearrangements that result in ALK fusion proteins. ALK fusion proteins are constitutively active and induce downstream signaling pathways including Ras/Raf/MEK/ERK1/2, JAK/STAT, PI3K/Akt, and PLC-γ [31] that promote cancer progression [32].

The targets of p53 , Neurotrophin receptor kinases, ALK and MYCN, whether accessing genes or initiating survival or death pathways are somewhat defined. Signaling pathways associated with MYCN, p53, neurotrophin receptor kinases, and ALK have either been shown to intersect with or have the potential to intersect with calcium signaling pathways. Other signal transduction pathways that intersect with calcium signaling include PI3K/Akt and Focal Adhesion Kinase. Calcium signaling in NB is becoming increasingly important, as more studies reveal novel mechanisms whereby calcium regulates NB proliferation, apoptosis, cell migration and invasion. This review will focus on these calcium-mediated processes.

2. Calcium signaling pathways

The divalent cation calcium is a unique ubiquitous messenger. In health, calcium executes a vast spectrum of processes via precise mechanisms, and conveys fast responses such as synaptic vesicle fusion, muscle contraction or long term changes that affect the regulation of genes and a change in overall cell physiology.

Calcium can easily precipitate with the phosphate group of adenosine triphosphate (ATP) generating an insoluble molecule and creating a toxic environment. In order to use ATP as an energy carrier and an anionic messenger, evolution had to unravel mechanisms that guarantee tight control over the presence of calcium. Under energy consumption, calcium is chelated, pumped, transported and exchanged leading to strong intracellular compartmentalization and a 10 to 20,000 fold concentration gradient from the cytoplasm to the extracellular space. The chemical features of calcium combined with the resulting spatial separation favored it as a potent intracellular messenger. Further, the evolution of calcium binding motifs like the EF hand or C2 domain permit introduction of positive charges that affect the conformation of the protein, thereby generating a molecular switch that is triggered by calcium [33].

Despite the discovery of the tissue specific localization of calcium-permeable channels in non-excitable cells, our knowledge of the regulation of some of these channels are still limited, with even less understanding of their role in the physiology of diseases.

Store operated calcium entry (SOCE) also termed capacitative calcium entry is one of the striking calcium signaling cascades in which the molecular mechanism has been unraveled within the current millennium. The phenomenon of SOCE was first described in the 1980s when researchers were still lacking genetic approaches and therefore relied on - at the time - newly developed imaging and electrophysiological technologies. Subsequently, a model was proposed "by which activation of surface membrane receptors causes sustained Ca^{2+} entry into cells from the extracellular space" [34]. It was further concluded that this calcium influx was driven by the emptying and filling of intracellular calcium pools, that also was dependent on the intracellular messenger inositol (1,4,5) trisphosphate. The biophysical properties, in particular the recordings of the tiny calcium selective current at the border of electrophysiological noise, were later more precisely defined in mast cells and termed I_{CRAC} (Calcium Release Activated Current) [35]. It wasn't until 2005 when STIM1 was identified as being the ER residing calcium sensor that activates I_{CRAC} by translocating to the plasma membrane [36,37]. Just a year later, ORAI was identified as the pore-containing CRAC channel [38–40], which was revealed by combing a linkage analysis of genes from patients with a hereditary form of severe combined immune deficiency syndrome (SCID) with a genome wide RNAi screen. In T cells, ORAI1 mediated sustained calcium elevation promotes dephosphorylation by the calmodulin-dependent protein phosphatase calcineurin. In the case of SCID patients, ORAI mutations lead to severe impairment in NFAT-dependent gene activation. Since then, several ER-residing proteins have been identified that either directly interfere with the STIM complex or otherwise modulate ER calcium homeostasis thereby affecting SOCE [41]. Among the components of SOCE so far only CHERP (calcium homeostasis endoplasmic reticulum protein) has been reported to impact NB malignancy [42].

The Transient Receptor Potential Melastatin (TRPM) Family Member 2 (TRPM2) is one of the 3 known channels that functions as an ion conducting channel and contains an intrinsic ADP-ribose pyrophosphatase domain [43]. TRPM2 shares homology with the NUDT9 domain in its C-terminal region, and thereby combines channel function with gating through the enzymatic domain of the channel. TRPM2 conducts mono and divalent cations nonspecifically, the resulting current voltage (IV) relationship under voltage clamp conditions is linear and reverses at 0 mV [44]. Leak currents share similar IV relationships and therefore currents can easily be misinterpreted under patch clamp conditions. Single channel and permeation studies as well as recordings of activation kinetics can be useful tools to distinguish TRPM2 mediated currents from secondary calcium activated currents that may occur despite the use of TRPM2 knock out models [45]. TRPM2 is gated by ADP Ribose and calcium [46]. Recently, a physiological role for TRPM2 was revealed. It was found to play a role as a hypothalamic heat sensor that mediates responses to temperatures above 37 °C, and may play a role in thermo protection against fever [47]. Further, TRPM2 activation is strongly tied to reactive oxygen species (ROS) sensing and other signals that lead to increases in intracellular ADPR levels, a function that may be critical in the cells of the innate immune system where TRPM2 is abundantly expressed [48]. Interestingly, TRPM2 splice variants have been identified that activate metabolic pathways [49], mobilize calcium across the plasma membrane and induce calcium release from lysosomes [50,51].

MYCN is a prognostic indicator for high risk NB. In an effort to understand whether the transcription factor MYCN modulates the expression of mediators of calcium signaling, a transcript analysis was performed, and the results identified TRPM7 among others as being responsive to MYCN levels [52,53]. TRPM7 is a calcium, magnesium and zinc conducing channel with a C-terminal S/T kinase [54]. In NB TRPM7 expression positively correlates with MYCN copy number [52,53]. In a NB cell line model of MYCN overexpression (TET-21 N) [55], tet-induced suppression also indicated TRPM6 as being correlative with MYCN levels [52]. However, MYCN does not transcriptionally target TRPM6 or TRPM7 directly [53]. Regulation of TRPM7 may occur indirectly, possibly through polyamine metabolism. MYCN regulates the expression of ornithine decarboxylase (ODC), the rate limiting enzyme of polyamine metabolism [56], pharmacologically targeting ODC using DFMO or genetically using ODC specific siRNA reduced intracellular polyamine levels and decreased MYCN-induced TRPM7 expression, an effect that was reversed by supplementing the culture media with exogenous polyamines. Alternatively, TRPM7 expression may be regulated indirectly through unknown intermediate transcription factors that are yet to be identified. TRPM7 and its closest homologue TRPM6 are ion channels that contain an intrinsic alpha-like kinase domain. The kinase domain of TRPM6 and TRPM7 may be cleaved and translocated throughout the cytoplasm or to the nucleus where it has been shown to phosphorylate myosin IIA and histones and regulate motility and gene expression, respectively [57,58].

It should also be noted that calcium channels may interact with each other either directly or through the regulation of the membrane potential in order to shape the overall extent of calcium flux [59]. In particular for SOCE, it has been reported that inhibition of TRPM7 activity reduces store-operated calcium entry likely through its kinase domain in a chicken B cell line [60].

How these regulations precisely impact the malignant progression of NB would require further studies as TRPM7 and the components of SOCE, SARAF, CHERP and SERCA3 (Fig. 1B, C and E) are at least indirectly transcriptionally regulated by MYCN.

The gating mechanisms of several of the TRP channels are still not fully understood [61,62]. Recent studies identified several calcium transporters and calcium regulator proteins that regulate or play a role in regulating NB progression. Interestingly, there have only been few research groups that investigated the role of calcium-permeable ion channels in NB in vivo. Using xenograft mouse models Dr. Barbara Miller's group identified a role for TRPM2 in NB progression, and Dr. Frank Van Leeuwen's group identified a role for TRPM7 in NB progression. Currently, no studies have been published investigating the role of calcium channels in NB utilizing the TH-MYCN transgenic model [63] or the recently established LSL-*MYCN*:Dbh-iCre model [64]. Furthermore, there have been no clinical studies investigating ion channels or calcium signaling proteins in NB. The R2 Genomic Analysis and Visualization Platform allows access and analysis of transcriptomic and RNAseq data from NB Patient tumors. R2 therefore presents a critical tool that helps to bridge the current gap in our understanding of the impact of calcium signaling on the pathophysiology of NB and allows to study specific ion channels and/or regulators of the calcium signaling machinery and their role in Neuroblastoma tumorigenesis and progression.

3. Genomic analysis of channels and regulators of the calciosome: Impact on Neuroblastoma malignant progression?

In order to overcome the lack of published data from preclinical and clinical studies investigating the role of calcium signaling proteins in NB progression, the use of RNAseq analysis may prove to be a powerful tool to identify the molecular components of calcium signaling pathways that promote NB progression. Further, in-silico analysis of patient-derived data provides the pathophysiologically relevant rationale that may be used to guide preclinical research in a cost efficient way.

In order to understand whether the calcium signaling machinery is altered at the transcriptional level in NB patient tumors, an in-silico analysis of channels and regulators of the calciosome was performed. We analyzed TRP channels, transporters and pumps critical for mitochondria functions, components of SOCE, organellar and plasma membrane channels that mediate the cell's overall calcium maintenance. All gene expression analyses were performed using the R2 platform (http://r2.amc.nl), using the SEQC RNAseq data set (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc = gse62564). Patients were sorted based on the expression of *GeneX* and subsequently divided into 2 groups according to the expression value of every patient. For every group separation (Higher or lower than the current *GeneX* expression), the logrank significance was calculated. The best p-value out of the sequence was then used to represent the final gene expression cut-off value for GeneX. This significance has been corrected for multiple testing by Bonferoni. For differential expression analyses, the *t*-test or ANOVA test was employed. For the survival analyses, the KaplanScan method, embedded in R2 was utilized to define the optimal group separation with respect to differences in survival.

Targets were evaluated for a correlation of expression with event free survival, INSS stage and MYCN amplification. In brief, Stage 1 neuroblastoma is defined as only primary tumor that is localized to one side of the body, and may be removed completely by surgery. Stage 2 neuroblastoma is similar to stage 1 but not all of the visible tumor could be removed by surgery, and the cancer has not infiltrated nearby lymph nodes but the lymph nodes may or may not contain neuroblastoma cells. Stage 3 neuroblastoma is defined as cancer that presents on both sides of the body and the cancer has not metastasized to distant parts of the body. In addition, the cancer may or may not have spread to nearby lymph nodes, and may not be completely removed by surgery. Stage 4 Neuroblastoma is divided into Stage 4 and 4 s. Stage 4 neuroblastoma is cancer that metastasized to distant parts of the body, including lymph nodes, bone or bone marrow, liver, skin, and other organs. Stage 4S neuroblastoma patients are less than 12 months old, have primary tumors localized to one side of the body, and present with metastasis limited to liver, skin and bone marrow, with less than 10% tumor cells in the bone marrow. Stage 4S is associated with favorable outcome compared to other neuroblastoma patients with metastatic disease [65].

An RNAseq data set (NB498) of nearly 500 patients from the SEQC consortium was used [66]. The analysis revealed that altered expression of components of SOCE, organellar and plasma membrane channels and pumps of calcium homeostasis as well as mitochondrial calcium transporters correlated with overall poor survival in NB.

The analysis indicated that several components of store-operated calcium entry were deregulated. The results showed that high expression of STIM2 correlated with low overall survival, stage 4 disease and MYCN gene amplification (Fig. 1A). STIM2 is an ER residing calcium sensor that can translocate to the plasma membrane and interact with ORAI channels thereby regulating gating and activation of store-operated calcium entry [67]. The role of STIM2 in physiology and cancer is less understood than its isoform STIM1 [68]. However, increased expression of STIM2 could enhance store-operated calcium entry. As SOCE was found to be uncoupled in differentiated NB cells, which sensitized the cells to cell death, it is possible that increased SOCE in advanced stage MYCN amplified NB promotes the survival of NB tumors and decreases patient overall survival. Similar results were observed with high expression of CHERP (Fig. 1C) and MCU (Fig. 1F), which correlated with low overall survival, stage 4 disease and MYCN gene amplification. CHERP promotes ER calcium release by modulating RyR-mediated calcium release and/or alternative splicing of IP3R1 [42]. This could modulate calcium signaling (e.g. SOCE), promote NB tumor survival, resulting in decreased patient overall survival. Conversely, high expression of SARAF correlated with high overall survival, early stage disease (stage 1 or 2) and non-amplified MYCN gene (Fig. 1B). SARAF is a negative regulator of STIM proteins, and consequently reduces SOCE [69]. High expression of SARAF could potentially decrease SOCE, reduce the growth and survival of NB tumors, resulting in high patient overall survival. Similar results were obtained with high expression of SERCA3 (Fig. 1E), RyR2 (Fig. 1D), PMCA4 (Fig. 1H) and NCLX (Fig. 1G), which correlated with high patient overall survival, stage 1 or 2 disease, and non-amplified MYCN gene.

MCU, a mitochondrial inner membrane uniporter mediates uptake of calcium into the MT, mitochondrial metabolism and cell death [70]. NCLX is a mitochondrial Na^{+}/Ca^{2+} exchanger that mediates MT calcium efflux [71]. Expression analysis of the of MCU and NCLX in NB indicates that the two MT Ca^{2+} regulators tightly regulates

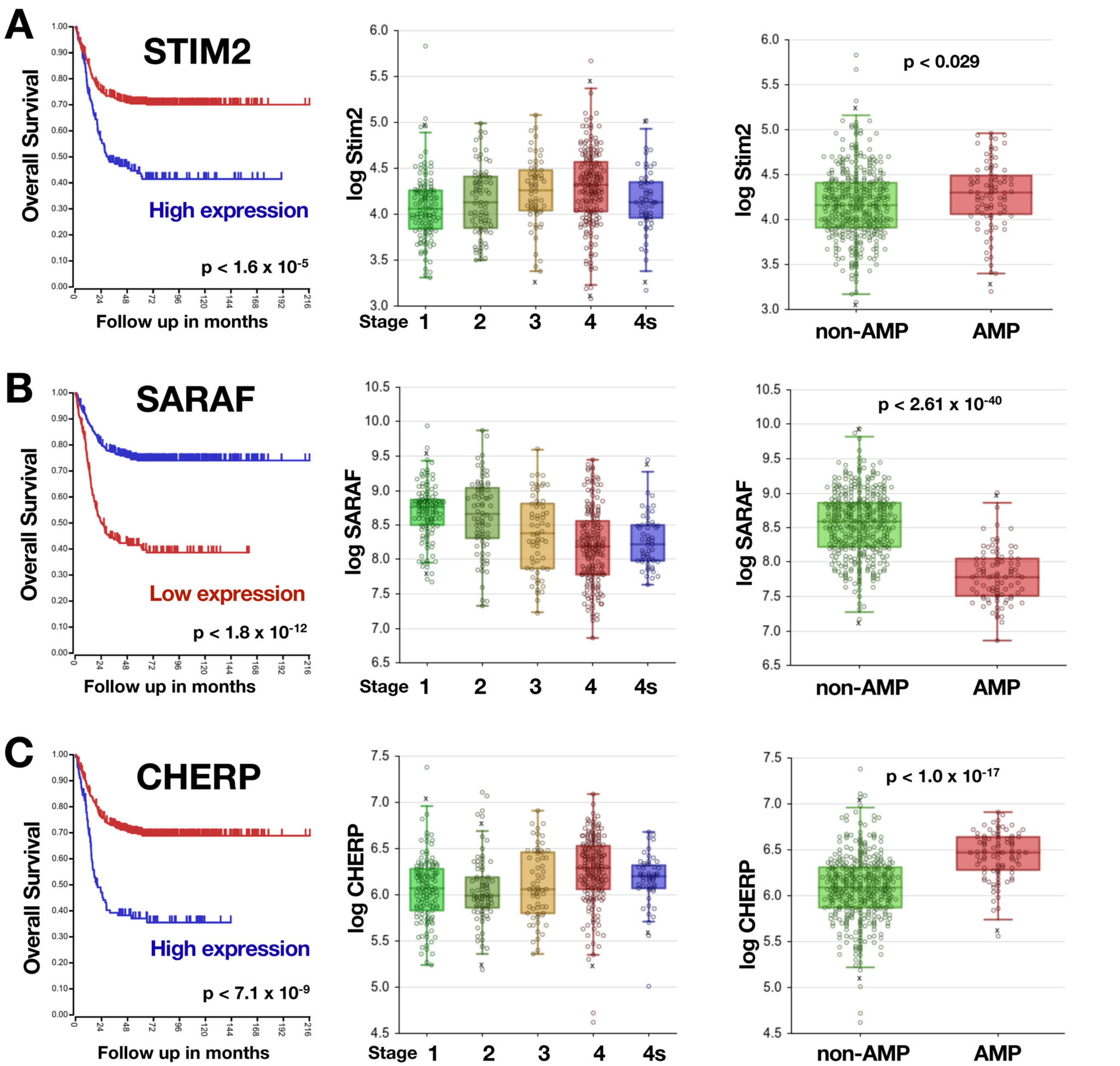

Fig. 1. The R2 NB498 RNAseq data set of nearly 500 patients from the SEQC consortium was used. A–H show expression analysis of regulators of calcium signaling with Kaplan survival plot, NB stage and MYCN correlation respectively. Color codes the expression levels of transcript (red: low expression, blue high expression). For Kaplan plots high and low expression were divided into **A** STIM2 (high n = 150; low n = 348), **B** SARAF (high n = 317; low n = 181), **C** CHERP (high n = 109; low n = 389), **D** RYR2 (high n = 335; low n = 163), **E** SERCA3 (high n = 444; low n = 54), **F** MCU (high n = 232; low n = 262), **G** NCLX (high n = 445; low n = 53), **H** PMCA4 (high n = 329; low n = 170), **I** TRPM2 (high n = 398; low n = 100), J TRPM7 (high n = 10; low n = 488). For NB stage analysis, Stage 1 NB n = 121, Stage 2 NB n = 78, Stage 3 n = 63, Stage 4 n = 183 and Stage 4 s n = 53, Anova p: A = $3.66e^{-5}$, B = $7e^{-18}$, C = $4.79e^{-6}$, D = $1.43e^{-7}$, E = $2.65e^{-8}$, F = $1.77e^{-3}$, G = $1.33e^{-9}$, H = $4.09e^{-25}$ I = $3.6e^{-6}$, J = 0.645. MYCN amplification plot, non amplified NB n = 401, MYCN amplified n = 92.

mitochondrial calcium uptake. MCU may function to shunt cytosolic calcium into the mitochondria to decrease cytosolic calcium levels or to maintain mitochondrial metabolism.

Further, analysis of TRPM2 expression in this tumor set revealed that expression of the channel negatively correlated with survival, high risk Neuroblastoma stage 4 and MYCN amplification (Fig. 1I). As previously reported by Zhang et al. [52] and Lange, Koomoa [53] using different patient derived data sets, TRPM7 expression also correlated with poor survival in the current data set. However the small cohort of TRPM7 high expressions data (n = 10) versus low TRPM7 (n = 488) do no indicate a sufficient impact on the TRPM7 expression averages that are depicted in the figure of Neuroblastoma stage separation. Further, the data set from NB patient samples only showed a trend of elevated expression of TRPM7 in MYCN amplified NB (Fig. 1J).

The correlation of calcium signaling proteins with clinical outcomes derived from NB patient tumor analysis provides evidence that components of the calcium signaling network may be involved in NB tumor progression and patient survival.

The mechanisms whereby these calcium signaling proteins may regulate NB progression and patient outcome remain largely unknown. However, there are several studies that showed how calcium signaling regulates NB processes, such as cell cycle progression, cell death, cell migration and differentiation, and will be the focus of the review in the following sections.

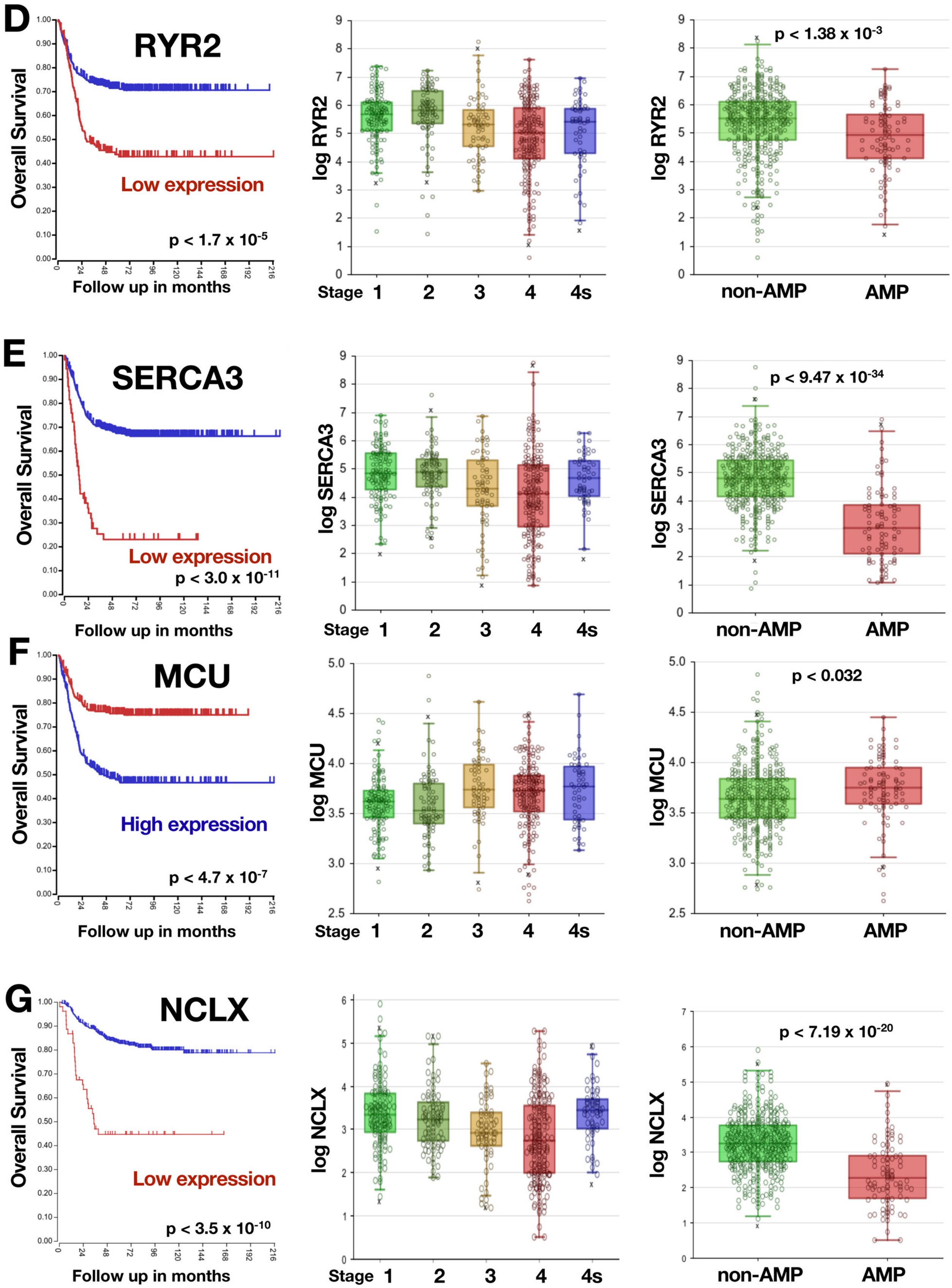

Fig. 1. (*continued*)

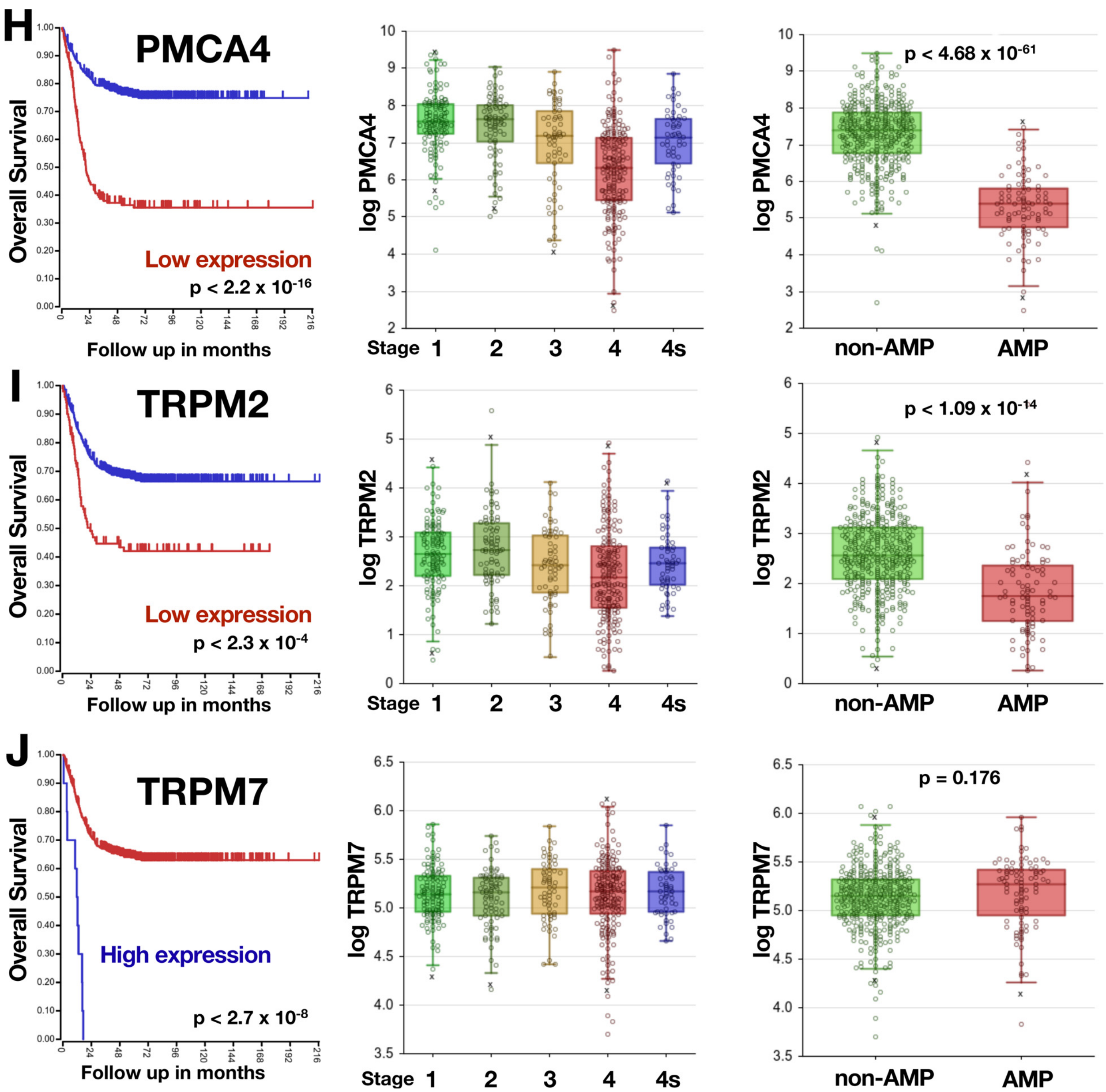

Fig. 1. (*continued*)

4. Role of calcium signaling in regulating neuroblastoma cell cycle progression

Cells progress through a series of phases that are characterized by specific events in order to duplicate itself, a process called cell division. The G0 phase is when the cell is quiescent and stops dividing. In the G1 phase of the cell cycle, the cell prepares for DNA synthesis by increasing the number of organelles, proteins and cell size. The cell then enters the S phase and replicates its DNA. In the G2 phase, the cell prepares for mitosis by increasing the synthesis of proteins required for mitosis. During the M phase, the cell enters and completes mitosis. At the end of each phase of the cell cycle, there are check point proteins that regulates the successful completion of the events in each phase of the cell cycle before progressing into the next phase.

Cell cycle progression is regulated by protein complexes made up of cyclins and cyclin dependent kinases (CDK) [72]. These protein complexes are in turn regulated by CDK activating kinases and CDK inhibitor proteins. Calcium regulates the expression and activation of cell cycle protein complexes directly or through activation of calmodulin (CaM) or calcineurin (CaN). Store-operated calcium and changes in cytosolic calcium via other calcium transporters regulate the progression through the different phases of the cell cycle, and play an important role in the transition through cell cycle checkpoints [73]. Previous studies showed that calcium oscillations or store-operated calcium entry and recycling of calcium to extracellular space or intracellular calcium stores is increased at the G1/S transition [74]. The increased calcium modulates the expression and activation of cell cycle protein complexes, and prepares the cell for DNA replication. In addition, CDK4/cyclin D1 activation leads to inactivation of retinoblastoma Rb1, which is the main inhibitor of DNA synthesis [75]. Upon completion of DNA replication in the S phase, calcium entry via store-operated calcium channels is reduced at the G2/M transition. Cytosolic and nuclear calcium was also shown to regulate progression through the different phases of mitosis. However, whether the calcium regulation of cell cycle described above also occurs in NB remain relatively unknown.

The sarco-endoplasmic reticulum calcium ATPase (SERCA) was shown to play a role in regulating cell cycle progression. Indeed, inhibition of SERCA via thapsigargin treatment, a SERCA inhibitor with high selectivity, induced entry into G0 phase and resulted in quiescence of SH-SY5Y NB cells [76]. Furthermore, a recent study showed that the calcium homeostasis endoplasmic reticulum protein (CHERP) plays a role in regulating NB cell cycle progression [42]. They showed that suppression of CHERP using siRNA induced G0/G1 cell cycle arrest and reduced the expression of CDK2 and Cyclin E.

5. The role of calcium signaling in Neuroblastoma cell death

There are different types of cell death. Apoptosis is one type of controlled or programmed cell death that is highly regulated by specific cues and signals, coming either from within the cell (intrinsic), or from the outside of the cell (extrinsic). Extrinsic apoptosis involves the binding of a ligand to a cell death receptor at the plasma membrae (e.g. TNF receptor or Fas receptor), activation of caspases and induction of apoptosis. Intrinsic apoptosis involves targeting mitochondria which may lead to opening of the mitochondrial permeability transition pore and release of mitochondrial proteins that promote activation of caspases and PARP followed by subsequent degradation of cellular components and cell death [77].

Calcium is tightly controlled in a dynamic fashion to elicit specific cellular responses to different stimuli. As mentioned earlier, ER calcium signaling plays a critical role in regulating fundamental physiological processes including metabolism, migration, cell death and differentiation. Store-operated calcium entry maintains calcium stores such as ER, which is important for cell survival. ER-mitochondrial calcium signaling regulates metabolism and cell death. Cellular calcium homeostasis is dependent on fine-tuned regulation of inter-organelle calcium signaling and signaling between organellar and plasma membrane calcium channels [78].

Fingolimod (FTY720) is an FDA approved drug for the treatment of multiple sclerosis [79]. Previous studies have shown that FTY720 targets TRPM7 channel and reduces its channel activity [80,81]. In addition, FTY720 was also shown to inhibit the TRPM7 kinase function, as shown by decreased phosphorylation of histone H3 and myosin IIa. Through a separate pathway, FTY720 induced NB cell death by disruption of mitochondrial membrane potential and calcium overload [81]. These studies suggest that TRPM7 may regulate NB cell death through a process that involves intracellular calcium signaling, as well as TRPM7 channel and kinase activities.

In NB cell lines, 2 splice variants of TRPM2 have been reported to mediate antagonistic functions. Overexpression of a short variant of TRPM2 (TRPM2-S) in SH-SY-5Y NB cells increased cell proliferation and ROS-induced susceptibility to cell death compared to its long variant counterpart (TRPM2-L). Further expression of TRPM-S in NB xenografts models reduced tumor mass and overall sensitivity to Doxorubicin was increased when compared to TRPM2-L [49]. TRPM2 depleted NB cell lines presented altered mitochondrial energetics as well as morphological changes when challenged with antineoplastic drugs [82]. Recently, H_2O_2 was shown to induce cell death through a TRPM2-mediated process. Interestingly, it was reported that H2O2 induced TRPM2-dependent delayed cell death through a positive feedback mechanism that involved an increase in intracellular zinc levels, increased mitochondrial zinc, mitochondria fission and lysosomal dysfunction [83–85].

Finally, recent studies have shown that chemotherapeutic drugs used to treat NB also regulates calcium signaling. Cisplatin and topotecan have shown to induce apoptosis, which was accompanied by increased intracellular free calcium over time. The cytotoxic effects of these drugs are mediated, at least in part, by calcium regulating proteins. Studies have shown that the expression of several calcium regulating proteins are differentially regulated with these chemotherapeutic drugs, including inositol triphosphate receptors (IP3R1 and IP3R3) and ryanodine receptors (RyR1 and RyR3) [86].

These studies suggest that calcium plays a critical role in regulating cell death. Therefore, targeting specific calcium signaling pathways may sensitize NB to the cytotoxic effects of chemotherapeutic drugs, and calcium regulating proteins may prove to be promising targets for the development of more effective drugs in the future.

6. The role of calcium signaling in Neuroblastoma cell migration

Cell migration is a process whereby a series of events occur in a highly coordinated fashion to allow the movement of a cell in response to a mechanical or chemical signal. This process involves formation and degradation of adhesion points at the leading and trailing edges of the cell, respectively. Concurrently, there is restructuring of cytoskeletal proteins to allow movement of the cell body towards the leading edge of the cell. Many of the processes involved in cell migration is regulated by calcium [33,87].

There have been several studies that investigated the role of calcium signaling in NB cell migration. One of the most predominant calcium channels that was found to regulate NB cell migration is TRPM7 [53,88]. Initial studies using an over-expression model of wild-type or kinase-dead TRPM7 showed that functional TRPM7 channel and kinase domains were required for phosphorylation of Myosin IIA, resulting in relaxation of actomyosin cytoskeleton and podosome formation [58,89,90]. Another study showed that TRPM7 promotes the formation of calcium hotspots during cell migration that regulates directional movement [88]. In addition, TRPM7 may regulate invadosome formation and cell migration independent of extracellular calcium and may be predominantly mediated by TRPM7's kinase activity. While there is very little information regarding the role of calcium in regulating NB cell migration, these studies show that TRPM7 plays a critical role in promoting NB cell migration.

7. The role of calcium signaling in Neuroblastoma differentiation

The histological grade of NB differentiation is used as a prognostic factor and to determine post-relapse survival, albeit to a lesser extent than other prognostic indicators (e.g. MYCN amplification, age at diagnosis, etc). Treatments that lead to differentiation of NB tumor cells, such as 9-cis retinoic acid (9cRA) are used after high-dose chemotherapy to prevent relapse and tumor recurrence [91,92]. Interestingly, 9cRA treatment have different effects on cellular morphology, calcium signaling and expression of calcium modulating proteins in different subpopulations of NB cells. A recent study showed that N-type NB cells treated with 9cRA had increased neurites and more neuronal-like morphology, increased expression of Bcl2 (anti-apoptotic protein), reduced ER calcium release and SOCE, which corresponded with lower expression of Stim1 and Orai1 [93]. 9cRA treatment of S-type NB cells resulted in more flattened glial-like morphology, decreased Bcl2 expression, increased ER calcium release but decreased SOCE and reduced expression of Stim1 and Orai1. I-type NB cells showed a mixture of effects on ER calcium release and SOCE upon 9cRA treatment. The more N-type cells in I-type NB cell lines had increased ER calcium release upon 9cRA treatment, compared to control untreated cells. However, the SOCE levels were similar in differentiated and control cells. Conversely, S-type cells in I-type NB cell lines had no change ER calcium release upon 9cRA treatment, but had increased SOCE upon 9cRA treatment compared to control untreated cells. Interestingly, upon withdrawal of 9cRA, N-type NB cells reverted to pre-treatment undifferentiated state, but S-type NB cells maintained differentiated state [94]. While there are few studies examining the role of calcium on NB differentiation, it is clear that calcium plays a critical role in regulating this process. The finding that differentiating drugs (e.g. 9cRA) induce varying effects on morphology, calcium signaling and expression of calcium regulating proteins in the different NB subpopulations bring up an as yet unanswered question of whether or not the calcium signaling that promotes NB progression are regulated differently in different NB subpopulations.

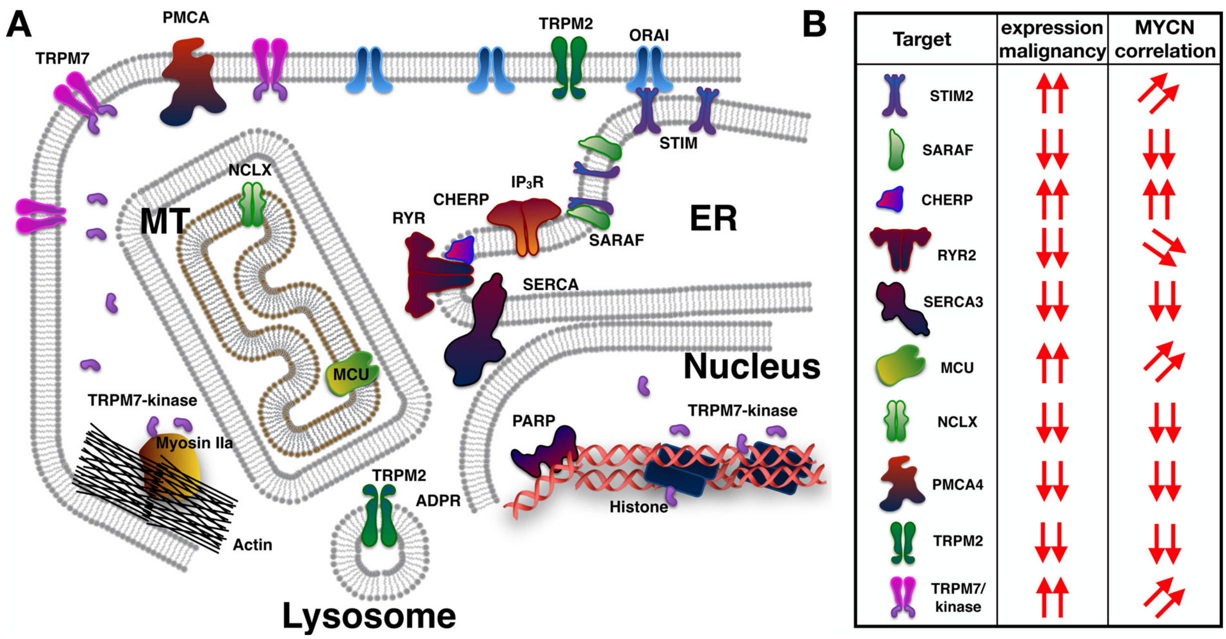

Fig. 2. Schematic view of channels pumps regulators and enzymatic targets that may play a role in the malignant progression of Neuroblastoma (**A**). Indication of transcriptional regulation of targets of calcium signaling in correlation to malignant progression (expression malignancy) as well as correlation of MYCN amplification in NB. Arrows indicate level of transcriptional regulation (**B**).

8. Summary

Neuroblastoma (NB) accounts for approximately 15% of cancer-related deaths in children. There is accumulating evidence from research on NB, as well as other cancers, that point to calcium signaling proteins as potential targets for novel treatments or adjunct treatments. TRPM2 has been shown to play a role in regulating ROS response and cell death in NB, through a mechanism that involves TRPM2-mediated influx of calcium from the extracellular environment and calcium release from lysosomes. TRPM7 has been shown to play a role in regulating several different events involved in NB cell migration, through a mechanism that involves TRPM7-mediated calcium entry and TRPM7 kinase activity. Interestingly, there are numerous reports of the role of intracellular calcium signaling and store-operated calcium entry in regulating NB progression, including but not limited to, cell cycle, cell death, cell migration, and differentiation. However, very little evidence exists that identifies the key molecular components involved in regulating these processes.

The discovery of immunotherapy and drugs that target validated cancer biomarkers have fundamentally transformed current approaches to cancer therapy [12,95]. However, we have yet to validate ion channels and calcium channel regulators as biomarkers for cancers, in particular NB. This is partly due to the fact that the expression and activity of channels are not homogenous among cell populations, which suggest intrinsic differences between subpopulations of cells and micro environmental signaling that may attenuate or prevent cell death, enhance cell proliferation, promote uncontrolled cell division, induce angiogenesis and multidrug resistance, or other processes involved in malignant progression. Further, the analysis of the function of an ion channel is tedious, requires electrophysiological recordings, and cannot efficiently be conducted in a high content setting with the sensitivity required for endogenous ion channels. Current literature demonstrates a role for TRPM2 and TRPM7 in NB progression, as well as calcium release from intracellular stores and SOCE in NB progression. However, the precise molecular components of calcium release and SOCE in NB have yet to be identified, and the mechanism whereby calcium release and SOCE regulates NB progression remain unknown. Overall, calcium release and SOCE may be key processes involved in NB progression. TRPM7 may enhance SOCE by facilitating the refilling of calcium into the ER. TRPM2 may modulate the calcium signaling involved in NB progression. Fig. 2 is a schematic illustration of calcium signaling targets that have been implicated to play are role in the malignant progression of Neuroblastoma.

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References

[1] J.M. Maris, M.D. Hogarty, R. Bagatell, S.L. Cohn, Neuroblastoma, Lancet 369 (2007) 2106–2120, https://doi.org/10.1016/S0140-6736(07)60983-0.
[2] A.T. Look, F.A. Hayes, R. Nitschke, N.B. McWilliams, A.A. Green, Cellular DNA content as a predictor of response to chemotherapy in infants with unresectable neuroblastoma, N. Engl. J. Med. 311 (1984) 231–235, https://doi.org/10.1056/NEJM198407263110405.
[3] B.R. Oppedal, I. Storm-Mathisen, S.O. Lie, P. Brandtzaeg, Prognostic factors in neuroblastoma. Clinical, histopathologic, and immunohistochemical features and DNA ploidy in relation to prognosis, Cancer 62 (1988) 772–780.
[4] G.M. Brodeur, R.C. Seeger, M. Schwab, H.E. Varmus, J.M. Bishop, Amplification of N-myc in untreated human neuroblastomas correlates with advanced disease stage, Science 224 (1984) 1121–1124.
[5] G.M. Brodeur, Neuroblastoma: biological insights into a clinical enigma, Nat. Rev. Cancer 3 (2003) 203–216, https://doi.org/10.1038/nrc1014.
[6] K.A. Cole, J.M. Maris, New strategies in refractory and recurrent neuroblastoma: translational opportunities to impact patient outcome, Clin. Cancer Res. 18 (2012) 2423–2428, https://doi.org/10.1158/1078-0432.CCR-11-1409.
[7] F. Berthold, B. Hero, Neuroblastoma: current drug therapy recommendations as part of the total treatment approach, Drugs 59 (2000) 1261–1277.
[8] V. Castel, E. Grau, R. Noguera, F. Martinez, Molecular biology of neuroblastoma, Clin. Transl. Oncol. 9 (2007) 478–483.
[9] S.L. Cohn, et al., The International Neuroblastoma Risk Group (INRG) classification system: an INRG Task Force report, J. Clin. Oncol. 27 (2009) 289–297, https://doi.org/10.1200/JCO.2008.16.6785.
[10] R. Spitz, et al., Favorable outcome of triploid neuroblastomas: a contribution to the

special oncogenesis of neuroblastoma, Cancer Genet. Cytogenet. 167 (2006) 51–56, https://doi.org/10.1016/j.cancergencyto.2005.09.001.
[11] R. Spitz, et al., Oligonucleotide array-based comparative genomic hybridization (aCGH) of 90 neuroblastomas reveals aberration patterns closely associated with relapse pattern and outcome, Genes Chromosomes Cancer 45 (2006) 1130–1142, https://doi.org/10.1002/gcc.20376.
[12] D.A. Morgenstern, S. Baruchel, M.S. Irwin, Current and future strategies for relapsed neuroblastoma: challenges on the road to precision therapy, J. Pediatr. Hematol. Oncol. 35 (2013) 337–347, https://doi.org/10.1097/MPH.0b013e318299d637.
[13] D. Sheiness, J.M. Bishop, DNA and RNA from uninfected vertebrate cells contain nucleotide sequences related to the putative transforming gene of avian myelocytomatosis virus, J. Virol. 31 (1979) 514–521.
[14] N.E. Kohl, et al., Transposition and amplification of oncogene-related sequences in human neuroblastomas, Cell 35 (1983) 359–367.
[15] M.M. Nau, et al., L-myc, a new myc-related gene amplified and expressed in human small cell lung cancer, Nature 318 (1985) 69–73.
[16] G.M. Brodeur, Neuroblastoma: clinical significance of genetic abnormalities, Cancer Surv. 9 (1990) 673–688.
[17] D.S. Rickman, J.H. Schulte, M. Eilers, The expanding world of N-MYC-driven tumors, Cancer Discov. 8 (2018) 150–163, https://doi.org/10.1158/2159-8290.CD-17-0273.
[18] P.S. Cohen, R.C. Seeger, T.J. Triche, M.A. Israel, Detection of N-myc gene expression in neuroblastoma tumors by in situ hybridization, Am. J. Pathol. 131 (1988) 391–397.
[19] D.N. Shapiro, et al., Detection of N-myc gene amplification by fluorescence in situ hybridization. Diagnostic utility for neuroblastoma, Am. J. Pathol. 142 (1993) 1339–1346.
[20] K. Campbell, et al., Association of MYCN copy number with clinical features, tumor biology, and outcomes in neuroblastoma: a report from the Children's Oncology Group, Cancer 123 (2017) 4224–4235, https://doi.org/10.1002/cncr.30873.
[21] R.C. Seeger, et al., Association of multiple copies of the N-myc oncogene with rapid progression of neuroblastomas, N. Engl. J. Med. 313 (1985) 1111–1116, https://doi.org/10.1056/NEJM198510313131802.
[22] C.L. Hsu, et al., Unveiling MYCN regulatory networks in neuroblastoma via integrative analysis of heterogeneous genomics data, Oncotarget 7 (2016) 36293–36310, https://doi.org/10.18632/oncotarget.9202.
[23] D.P. Lane, L.V. Crawford, T antigen is bound to a host protein in SV40-transformed cells, Nature 278 (1979) 261–263.
[24] K.H. Vousden, C. Prives, Blinded by the light: the growing complexity of p53, Cell 137 (2009) 413–431, https://doi.org/10.1016/j.cell.2009.04.037.
[25] C. Giorgi, et al., p53 at the endoplasmic reticulum regulates apoptosis in a Ca2+-dependent manner, Proc. Natl. Acad. Sci. U. S. A. 112 (2015) 1779–1784, https://doi.org/10.1073/pnas.1410723112.
[26] G.M. Brodeur, Molecular pathology of human neuroblastomas, Semin. Diagn. Pathol. 11 (1994) 118–125.
[27] G.M. Brodeur, et al., Trk receptor expression and inhibition in neuroblastomas, Clin. Cancer Res. 15 (2009) 3244–3250, https://doi.org/10.1158/1078-0432.CCR-08-1815.
[28] Y. Mizoguchi, A. Monji, J. Nabekura, Brain-derived neurotrophic factor induces long-lasting Ca2+-activated K+ currents in rat visual cortex neurons, Eur. J. Neurosci. 16 (2002) 1417–1424.
[29] N.K. Cheung, M.A. Dyer, Neuroblastoma: developmental biology, cancer genomics and immunotherapy, Nat. Rev. Cancer 13 (2013) 397–411, https://doi.org/10.1038/nrc3526.
[30] Y.P. Mosse, Anaplastic lymphoma kinase as a cancer target in pediatric malignancies, Clin. Cancer Res. 22 (2016) 546–552, https://doi.org/10.1158/1078-0432.CCR-14-1100.
[31] R. Roskoski Jr, Anaplastic lymphoma kinase (ALK): structure, oncogenic activation, and pharmacological inhibition, Pharmacol. Res. 68 (2013) 68–94, https://doi.org/10.1016/j.phrs.2012.11.007.
[32] S. Fransson, et al., Intragenic anaplastic lymphoma kinase (ALK) rearrangements: translocations as a novel mechanism of ALK activation in neuroblastoma tumors, Genes Chromosomes Cancer 54 (2015) 99–109, https://doi.org/10.1002/gcc.22223.
[33] D.E. Clapham, Calcium signaling, Cell 131 (2007) 1047–1058, https://doi.org/10.1016/j.cell.2007.11.028.
[34] J.W. Putney Jr, A model for receptor-regulated calcium entry, Cell Calcium 7 (1986) 1–12.
[35] M. Hoth, R. Penner, Depletion of intracellular calcium stores activates a calcium current in mast cells, Nature 355 (1992) 353–356, https://doi.org/10.1038/355353a0.
[36] J. Roos, et al., STIM1, an essential and conserved component of store-operated Ca2+ channel function, J. Cell Biol. 169 (2005) 435–445, https://doi.org/10.1083/jcb.200502019.
[37] J. Liou, et al., STIM is a Ca2+ sensor essential for Ca2+-store-depletion-triggered Ca2+ influx, Curr. Biol. 15 (2005) 1235–1241, https://doi.org/10.1016/j.cub.2005.05.055.
[38] S. Feske, et al., A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function, Nature 441 (2006) 179–185, https://doi.org/10.1038/nature04702.
[39] M. Vig, et al., CRACM1 is a plasma membrane protein essential for store-operated Ca2+ entry, Science 312 (2006) 1220–1223, https://doi.org/10.1126/science.1127883.
[40] S.L. Zhang, et al., Genome-wide RNAi screen of Ca(2+) influx identifies genes that regulate Ca(2+) release-activated Ca(2+) channel activity, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 9357–9362, https://doi.org/10.1073/pnas.0603161103.
[41] J.J. Lopez, et al., Molecular modulators of store-operated calcium entry, Biochim. Biophys. Acta 1863 (2016) 2037–2043, https://doi.org/10.1016/j.bbamcr.2016.04.024.
[42] D. Zhang, et al., Down-regulation of CHERP inhibits neuroblastoma cell proliferation and induces apoptosis through ER stress induction, Oncotarget 8 (2017) 80956–80970, https://doi.org/10.18632/oncotarget.20898.
[43] A.L. Perraud, et al., ADP-ribose gating of the calcium-permeable LTRPC2 channel revealed by Nudix motif homology, Nature 411 (2001) 595–599, https://doi.org/10.1038/35079100.
[44] I. Lange, R. Penner, A. Fleig, A. Beck, Synergistic regulation of endogenous TRPM2 channels by adenine dinucleotides in primary human neutrophils, Cell Calcium 44 (2008) 604–615, https://doi.org/10.1016/j.ceca.2008.05.001.
[45] S. Yamamoto, et al., TRPM2-mediated Ca2+influx induces chemokine production in monocytes that aggravates inflammatory neutrophil infiltration, Nat. Med. 14 (2008) 738–747, https://doi.org/10.1038/nm1758.
[46] Y. Huang, P.A. Winkler, W. Sun, W. Lu, J. Du, Architecture of the TRPM2 channel and its activation mechanism by ADP-ribose and calcium, Nature 562 (2018) 145–149, https://doi.org/10.1038/s41586-018-0558-4.
[47] K. Song, et al., The TRPM2 channel is a hypothalamic heat sensor that limits fever and can drive hypothermia, Science 353 (2016) 1393–1398, https://doi.org/10.1126/science.aaf7537.
[48] Y. Hara, et al., LTRPC2 Ca2+-permeable channel activated by changes in redox status confers susceptibility to cell death, Mol. Cell 9 (2002) 163–173.
[49] S.J. Chen, et al., A splice variant of the human ion channel TRPM2 modulates neuroblastoma tumor growth through hypoxia-inducible factor (HIF)-1/2alpha, J. Biol. Chem. 289 (2014) 36284–36302, https://doi.org/10.1074/jbc.M114.620922.
[50] I. Lange, et al., TRPM2 functions as a lysosomal Ca2+-release channel in beta cells, Sci. Signal. 2 (2009), https://doi.org/10.1126/scisignal.2000278 ra23.
[51] P. Wyrsch, C. Blenn, J. Bader, F.R. Althaus, Cell death and autophagy under oxidative stress: roles of poly(ADP-Ribose) polymerases and Ca(2+), Mol. Cell. Biol. 32 (2012) 3541–3553, https://doi.org/10.1128/MCB.00437-12.
[52] Z. Zhang, et al., N-Myc-induced up-regulation of TRPM6/TRPM7 channels promotes neuroblastoma cell proliferation, Oncotarget 5 (2014) 7625–7634, https://doi.org/10.18632/oncotarget.2283.
[53] I. Lange, D.L. Koomoa, MycN promotes TRPM7 expression and cell migration in neuroblastoma through a process that involves polyamines, FEBS Open Bio 4 (2014) 966–975, https://doi.org/10.1016/j.fob.2014.10.012.
[54] M.J. Nadler, et al., LTRPC7 is a Mg.ATP-regulated divalent cation channel required for cell viability, Nature 411 (2001) 590–595, https://doi.org/10.1038/35079092.
[55] W. Lutz, et al., Conditional expression of N-myc in human neuroblastoma cells increases expression of alpha-prothymosin and ornithine decarboxylase and accelerates progression into S-phase early after mitogenic stimulation of quiescent cells, Oncogene 13 (1996) 803–812.
[56] M.D. Hogarty, et al., ODC1 is a critical determinant of MYCN oncogenesis and a therapeutic target in neuroblastoma, Cancer Res. 68 (2008) 9735–9745, https://doi.org/10.1158/0008-5472.CAN-07-6866.
[57] G. Krapivinsky, L. Krapivinsky, Y. Manasian, D.E. Clapham, The TRPM7 chanzyme is cleaved to release a chromatin-modifying kinase, Cell 157 (2014) 1061–1072, https://doi.org/10.1016/j.cell.2014.03.046.
[58] K. Clark, et al., TRPM7, a novel regulator of actomyosin contractility and cell adhesion, EMBO J. 25 (2006) 290–301, https://doi.org/10.1038/sj.emboj.7600931.
[59] S. Saul, H. Stanisz, C.S. Backes, E.C. Schwarz, M. Hoth, How ORAI and TRP channels interfere with each other: interaction models and examples from the immune system and the skin, Eur. J. Pharmacol. 739 (2014) 49–59, https://doi.org/10.1016/j.ejphar.2013.10.071.
[60] M. Faouzi, T. Kilch, F.D. Horgen, A. Fleig, R. Penner, The TRPM7 channel kinase regulates store-operated calcium entry, J. Physiol. 595 (2017) 3165–3180, https://doi.org/10.1113/JP274006.
[61] V. Chubanov, S. Schafer, S. Ferioli, T. Gudermann, Natural and synthetic modulators of the TRPM7 channel, Cells 3 (2014) 1089–1101, https://doi.org/10.3390/cells3041089.
[62] J. Broertjes, J. Klarenbeek, Y. Habani, M. Langeslag, K. Jalink, TRPM7 residue S1269 mediates cAMP dependence of Ca2+ influx, PLoS One 14 (2019) e0209563, , https://doi.org/10.1371/journal.pone.0209563.
[63] W.A. Weiss, K. Aldape, G. Mohapatra, B.G. Feuerstein, J.M. Bishop, Targeted expression of MYCN causes neuroblastoma in transgenic mice, EMBO J. 16 (1997) 2985–2995, https://doi.org/10.1093/emboj/16.11.2985.
[64] K. Althoff, et al., A Cre-conditional MYCN-driven neuroblastoma mouse model as an improved tool for preclinical studies, Oncogene 34 (2015) 3357–3368, https://doi.org/10.1038/onc.2014.269.
[65] 65National Cancer Institute. Stages of Neuroblastoma, (2019) < https://www.cancer.gov/types/neuroblastoma/patient/neuroblastoma-treatment-pdq#_21 > (2019, March 28).
[66] S.M.-I. Consortium, A comprehensive assessment of RNA-seq accuracy, reproducibility and information content by the Sequencing Quality Control Consortium, Nat. Biotechnol. 32 (2014) 903–914, https://doi.org/10.1038/nbt.2957.
[67] J.W. Putney, et al., The functions of store-operated calcium channels, Biochim Biophys Acta Mol Cell Res 1864 (2017) 900–906, https://doi.org/10.1016/j.bbamcr.2016.11.028.
[68] Y.F. Chen, L.H. Chen, M.R. Shen, The distinct role of STIM1 and STIM2 in the regulation of store-operated Ca(2+) entry and cellular function, J. Cell. Physiol. 234 (2019) 8727–8739, https://doi.org/10.1002/jcp.27532.
[69] R. Palty, A. Raveh, I. Kaminsky, R. Meller, E. Reuveny, SARAF inactivates the store operated calcium entry machinery to prevent excess calcium refilling, Cell 149 (2012) 425–438, https://doi.org/10.1016/j.cell.2012.01.055.

[70] N. Nemani, S. Shanmughapriya, M. Madesh, Molecular regulation of MCU: implications in physiology and disease, Cell Calcium 74 (2018) 86–93, https://doi.org/10.1016/j.ceca.2018.06.006.
[71] M. Kostic, I. Sekler, Functional properties and mode of regulation of the mitochondrial Na(+)/Ca(2+) exchanger, NCLX, Semin. Cell Dev. Biol. (2019), https://doi.org/10.1016/j.semcdb.2019.01.009 [Epub ahead of print].
[72] M.B. Kastan, J. Bartek, Cell-cycle checkpoints and cancer, Nature 432 (2004) 316–323, https://doi.org/10.1038/nature03097.
[73] R. Hodeify, et al., J.A. Kozak, J.W. PutneyJr (Eds.), Calcium Entry Channels in Non-Excitable Cells, 2018, pp. 215–240.
[74] Y.W. Chen, Y.F. Chen, Y.T. Chen, W.T. Chiu, M.R. Shen, The STIM1-Orai1 pathway of store-operated Ca2+ entry controls the checkpoint in cell cycle G1/S transition, Sci. Rep. 6 (2016) 22142, https://doi.org/10.1038/srep22142.
[75] E.S. Knudsen, C. Buckmaster, T.T. Chen, J.R. Feramisco, J.Y. Wang, Inhibition of DNA synthesis by RB: effects on G1/S transition and S-phase progression, Genes Dev. 12 (1998) 2278–2292.
[76] C. Cecchi, et al., Interaction between acylphosphatase and SERCA in SH-SY5Y cells, Mol. Cell. Biochem. 211 (2000) 95–102.
[77] Y. Fuchs, H. Steller, Live to die another way: modes of programmed cell death and the signals emanating from dying cells, Nat. Rev. Mol. Cell Biol. 16 (2015) 329–344, https://doi.org/10.1038/nrm3999.
[78] L. Galluzzi, et al., Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell Death 2018, Cell Death Differ. 25 (2018) 486–541, https://doi.org/10.1038/s41418-017-0012-4.
[79] J. Chun, H.P. Hartung, Mechanism of action of oral fingolimod (FTY720) in multiple sclerosis, Clin. Neuropharmacol. 33 (2010) 91–101, https://doi.org/10.1097/WNF.0b013e3181cbf825.
[80] X. Qin, et al., Sphingosine and FTY720 are potent inhibitors of the transient receptor potential melastatin 7 (TRPM7) channels, Br. J. Pharmacol. 168 (2013) 1294–1312, https://doi.org/10.1111/bph.12012.
[81] I. Lange, I. Espinoza-Fuenzalida, M.W. Ali, L.E. Serrano, D.T. Koomoa, FTY-720 induces apoptosis in neuroblastoma via multiple signaling pathways, Oncotarget 8 (2017) 109985–109999, https://doi.org/10.18632/oncotarget.22452.
[82] L. Bao, et al., Depletion of the human ion channel TRPM2 in neuroblastoma demonstrates its key role in cell survival through modulation of mitochondrial reactive oxygen species and bioenergetics, J. Biol. Chem. 291 (2016) 24449–24464, https://doi.org/10.1074/jbc.M116.747147.
[83] X. An, et al., Increasing the TRPM2 channel expression in human neuroblastoma SH-SY5Y cells augments the susceptibility to ROS-Induced cell death, Cells 8 (2019), https://doi.org/10.3390/cells8010028.
[84] I. Hirschler-Laszkiewicz, et al., The human ion channel TRPM2 modulates neuroblastoma cell survival and mitochondrial function through Pyk2, CREB, and MCU activation, Am. J. Physiol. Cell Physiol. 315 (2018) C571–C586, https://doi.org/10.1152/ajpcell.00098.2018.
[85] X. Li, W. Yang, L.H. Jiang, Alteration in intracellular Zn(2+) homeostasis as a result of TRPM2 channel activation contributes to ROS-induced hippocampal neuronal death, Front. Mol. Neurosci. 10 (2017) 414, https://doi.org/10.3389/fnmol.2017.00414.
[86] A.M. Florea, et al., Calcium-regulatory proteins as modulators of chemotherapy in human neuroblastoma, Oncotarget 8 (2017) 22876–22893, https://doi.org/10.18632/oncotarget.15283.
[87] K.M. Mladinich, A. Huttenlocher, WRAMPing up calcium in migrating cells by localized ER transport, Dev. Cell 26 (2013) 560–561, https://doi.org/10.1016/j.devcel.2013.09.013.
[88] D. Visser, et al., TRPM7 triggers Ca2+ sparks and invadosome formation in neuroblastoma cells, Cell Calcium 54 (2013) 404–415, https://doi.org/10.1016/j.ceca.2013.09.003.
[89] K. Clark, et al., TRPM7 regulates myosin IIA filament stability and protein localization by heavy chain phosphorylation, J. Mol. Biol. 378 (2008) 790–803, https://doi.org/10.1016/j.jmb.2008.02.057.
[90] J. Middelbeek, et al., The TRPM7 interactome defines a cytoskeletal complex linked to neuroblastoma progression, Eur. J. Cell Biol. 95 (2016) 465–474, https://doi.org/10.1016/j.ejcb.2016.06.008.
[91] K.K. Matthay, et al., Treatment of high-risk neuroblastoma with intensive chemotherapy, radiotherapy, autologous bone marrow transplantation, and 13-cis-retinoic acid. Children's Cancer Group, N. Engl. J. Med. 341 (1999) 1165–1173, https://doi.org/10.1056/NEJM199910143411601.
[92] G. Cernaianu, et al., All-trans retinoic acid arrests neuroblastoma cells in a dormant state. Subsequent nerve growth factor/brain-derived neurotrophic factor treatment adds modest benefit, J. Pediatr. Surg. 43 (2008) 1284–1294, https://doi.org/10.1016/j.jpedsurg.2008.01.007.
[93] N. Bell, V. Hann, C.P. Redfern, T.R. Cheek, Store-operated Ca(2+) entry in proliferating and retinoic acid-differentiated N- and S-type neuroblastoma cells, Biochim. Biophys. Acta 1833 (2013) 643–651, https://doi.org/10.1016/j.bbamcr.2012.11.025.
[94] C.L. Whitworth, C.P.F. Redfern, T.R. Cheek, Differentiation-induced remodelling of store-operated calcium entry is independent of neuronal or glial phenotype but modulated by cellular context, Mol. Neurobiol. 56 (2019) 857–872, https://doi.org/10.1007/s12035-018-1112-y.
[95] J. Shohet, J. Foster, Neuroblastoma, BMJ 357 (2017) j1863, https://doi.org/10.1136/bmj.j1863.