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WT1/EGR1-mediated Control of STIM1 Expression and Function in Cancer Cells

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Abstract

There have been numerous publications linking Ca^{2+} signaling and cancer, however, a clear explanation for these differences has remained elusive. We recently identified the oncogenes/tumor suppressors Wilms Tumor Suppressor 1 (WT1) and Early Growth Response 1 (EGR1) as regulators of the expression of STIM1, an essential regulator of Ca^{2+} entry in non-excitable cells. The current review focuses on the literature defining both differential Ca^{2+} signaling and WT1/EGR1 expression patterns in 5 specific cancer subtypes: Acute Myeloid Leukemia, Wilms Tumor, breast cancer, glioblastoma and prostate cancer. For each tumor-type, we have assessed how specific changes in WT1 and EGR1 expression might contribute to aberrant Ca^{2+} homeostasis as well as the therapeutic potential of these observations.

2. Introduction

The extensive relationship between modulation of intracellular Ca^{2+} content and the control of cell proliferation (1–3), differentiation (4, 5) and death (6) has led to much examination into the relationship between Ca^{2+} signaling pathways and the onset and progression of tumorigenesis. The earliest evidence of differential control of Ca^{2+} signaling in cancer cells came from failed attempts to inhibit the proliferation of transformed mouse fibroblasts by removing extracellular calcium (7–9). This fact was initially interpreted to mean that cancer cells function in a Ca^{2+} -independent manner. However, the greater than 20,000 papers that have been published on the subject of Ca^{2+} signaling and homeostasis in cancer cells reveal a considerably more complex relationship between Ca^{2+} signaling and cancer. This is, perhaps, not at all surprising given both the complexity of the mechanisms in control of Ca^{2+} homeostasis and the variety of distinct diseases that the word “cancer” refers to. Therefore, this review will attempt to both summarize some of the key events leading to dysregulation of Ca^{2+} homeostasis in specific classes of cancer cells and define how this dysregulation could be used for therapeutic advantage.

2.1. Bcl2-mediated control of ER Ca^{2+} content in cancer cells

Loss of the ability to undergo apoptosis is one of the defining components of cancer. Perhaps the most critical family of proteins in control of apoptosis is the Bcl2 family proteins. This family consists of both anti-apoptotic (eg. Bcl2, Bcl-XL, Mcl-1) and pro-apoptotic members (eg. Bax, Bak); during transformation, a shift in the expression patterns towards the anti-apoptotic members of this family are often observed (10–12). Intriguingly, this shift is known to have significant effects on ER Ca^{2+} content (13–20). Although early investigations seemed to support the conclusion that Bcl2 directly releases ER luminal Ca^{2+}

(16, 17), subsequent studies have tended to support a modulatory role for Bcl-2. Hence, the primary mediator of receptor-induced Ca^{2+} transients is the inositol 1,4,5-triphosphate receptor (InsP_3R), which responds to phospholipase C-dependent production of InsP_3 by releasing Ca^{2+} into the cytoplasm from the ER. It has been shown that Bcl2, Bcl-XL and Mcl-1 directly bind to the InsP_3R , resulting in spontaneous activity under basal conditions, thereby leading to decreases in ER Ca^{2+} content (13–15). Furthermore, the proapoptotic proteins BAK and BAX counter this effect, decreasing InsP_3R activity and increasing ER Ca^{2+} content (20). Intriguingly, this relationship is reversed under agonist-stimulated conditions; in the presence of relatively high levels of InsP_3 , the effect of Bcl2- InsP_3R interactions is inhibition of ER Ca^{2+} release (18, 19). The net effect of these modulations of InsP_3R function may be to avoid large elevations in cytosolic Ca^{2+} concentration. Hence, the two distinct effects of Bcl2 modulations of InsP_3R activity likely work in concert to limit the amount of Ca^{2+} that can be released under stimulated conditions.

2.2. TRP cation channels function in cancer cells

Due to their tremendous diversity and wide expression (21), numerous investigations have been directed at identifying differences in the expression and/or function of members of the transient receptor potential (TRP) superfamily of cationic channels in diverse tumor types. While there are numerous examples of these types of changes, this relationship exhibits considerable cell-type specificity. For example, examination of melanoma metastases revealed complete loss of TRPM1 expression when compared with normal melanocytes (22), yet TRPV6 and TRPM8 are greatly upregulated in prostate cancer (23–25) and TRPC6 is dramatically upregulated in hepatoma (26). The case for TRPC6 as a promoter of tumorigenesis in liver is further supported by the fact that TRPC6 overexpression in Huh-7 (human hepatoma) cells causes an 80% increase in the rate of proliferation, while TRPC6-knockdown significantly decrease the rate of proliferation (26). Currently, the reasons why certain tumor types tend to regulate one TRP channel vs. another TRP channel remain a mystery. Nevertheless, these observations provide strong support for the idea that such a relationship does exist.

2.3. Molecular Mechanisms of Store-operated Ca^{2+} entry

The concept of store-operated Ca^{2+} entry (SOCE) was initially proposed in 1986 by Jim Putney (27), however, until recently the molecular mechanisms controlling SOCE were unknown. In 2005, 2 papers were published identifying STIM1 as a required component of this process (28, 29), followed in 2006 by 3 papers revealing a similar requirement for Orai1 (30–32). Over the last 4 years, considerable progress has been made defining how SOCE works (Fig 1) (33–38). Thus, both STIM1 and its mammalian homologue STIM2 are type 1A transmembrane proteins containing low Ca^{2+} affinity luminal EF hands (39); when ER Ca^{2+} content is high, their EF hands are bound to Ca^{2+} and the proteins are inactive (28, 38, 40). Decreases in ER Ca^{2+} concentration cause dissociation of Ca^{2+} from the STIM EF hands, resulting in a conformational change (40–42) that leads to STIM aggregation in regions of the ER adjacent to the PM (43–45), where they interact with and activate Orai1, the store-operated Ca^{2+} channel (35, 43, 44, 46–52). Despite the similarities in both their domain structure and general physiological roles, we and others have observed extensive differences in the activation characteristics of STIM1 and STIM2 (42, 53–56). Thus, the Ca^{2+} affinity of the STIM2 EF hand is at ~resting ER Ca^{2+} concentration, resulting in constitutive activation (35, 53, 54). However, sequences within the N-terminal tail of STIM2 control its rate of activation, thereby avoiding Ca^{2+} overload (56). There are also two mammalian homologues of Orai1 termed Orai2 and Orai3 which function similarly to Orai1 when overexpressed (57, 58), although the roles of the endogenous proteins remain undefined. Thus, while many questions remain, recent studies have led to considerable progress in the characterization of the molecular mechanisms of SOCE. Less clear is how

these proteins are regulated under both physiological and pathophysiological conditions. However, our recent investigations have provided an intriguing link between the expression of STIM1 and the oncogenes/tumor suppressors Early Growth Response 1 (EGR1) and Wilms Tumor Suppressor 1 (WT1) (59). Much of the current review focuses on describing the implications of these observations to cancer cell biology.

2.4. The zinc finger transcription factors WT1 and EGR1

The EGR family of zinc finger transcription factors consists of 4 closely related members (EGR1–4) that are rapidly, but transiently upregulated by a wide variety of extracellular stimuli including activation, growth and differentiation signals, tissue injury and apoptotic signals (60, 61). While EGR-binding elements have been identified in a vast panel of gene promoters of multiple classes, our group is primarily interested in the remarkable number of EGR-dependent genes involved in control of Ca^{2+} homeostasis. Thus, while we have shown that STIM1 transcription is directly regulated by EGR1 (59), others have shown that EGR1 negatively regulates the expression of the Sodium/ Ca^{2+} exchanger (62) and Calsequestrin (63). There is also evidence of EGR1-dependent control of SERCA2 expression (64–66), although we now believe this to be via an indirect mechanism (67). In contrast, the role of WT1, as a regulator of Ca^{2+} homeostasis was not investigated prior to our study revealing repression of STIM1 expression (59). Nevertheless, our observations are highly consistent with numerous investigations by other investigators revealing that WT1 represses EGR1-dependent gene expression (68–70).

Despite considerable structural similarity to EGR1, WT1 is not considered to be a member of the EGR family. This is primarily because, unlike EGR genes, WT1 is not generally responsive to growth factor stimulation; it is predominantly a developmentally regulated gene. A key feature of WT1 is the existence at least 2 sites for alternative splicing, resulting in 4 major splice variants (71). The first and most significant alternative splice donor site results in the addition of the amino acids KTS (Lys-Thr-Ser) between zinc fingers three and four (71). WT1 variants A and B, which lack this KTS site are the forms best described as transcription factors; the functions served by WT1 variants C and D (KTS+) is a subject of ongoing controversy. Whereas these proteins have been thought to function post-transcriptionally as RNA splicing proteins (71), KTS+ forms of WT1 have been reported to regulate gene transcription, albeit with distinct binding characteristics (72). The other major alternative splice donor site results in the inclusion of 17 amino acids in the middle of the protein in WT1 B and D, which is thought to regulate interactions between WT1 and cofactors (73).

3. WT1/EGR1 as oncogenes/tumor suppressors regulating Ca^{2+} homeostasis

In a recent investigation published in *J Biol Chem* (2010), we revealed that the expression of STIM1 was under the control of the transcription factors WT1 and EGR1 (59). Thus, either EGR1 knockdown or WT1 overexpression resulted in both loss of STIM1 expression and decreased SOCE. We further established that this regulation was direct by pulling down regions of DNA within 500 base pairs of the STIM1 transcriptional start site using either EGR1 or WT1 antibodies, a technique referred to as chromatin immunoprecipitation (ChIP). Finally, we revealed that WT1+ primary Wilms Tumor cells (representative of ~85% of Wilms Tumors) exhibit significant loss of both STIM1 expression and SOCE. However, dysregulated expression of EGR1 and/or WT1 is very common in multiple tumor types (74–85). Although a direct link between this dysregulation and Ca^{2+} homeostasis in these cell types has not been established, numerous clues exist in support of this concept, as outlined further below.

3.1. WT1 as a negative regulator of STIM1 expression in Wilms Tumor

Loss of WT1 expression due to deletion at 11p13 is closely linked to the formation of Wilms tumor, the most common peritoneal pediatric tumor, occurring 1/10,000 people (77, 79). However, this represents only a subset of Wilms Tumors, with approximately 80% of Wilms Tumors classified as “sporadic” and strongly expressing the transcriptional regulator (77, 79, 86). Due to the resistance that cells derived from bona-fide Wilms Tumors exhibit to growth in vitro, many early investigations of Wilms Tumor function were performed in non-Wilms Tumor cell types that exhibited key similarities to specific Wilms Tumor characteristics. A particularly intriguing example of this is the WT1-null G401 cell line, which was derived from a human rhabdoid tumor of the kidney (87). In work performed prior to the discovery of its role in control of SOCE, STIM1 was defined as a tumor suppressor in G401 cells and rhabdomyosarcoma (88, 89). While we cannot agree with the label of “tumor suppressor” for STIM1, we have now thoroughly examined Ca^{2+} homeostasis in G401 cells (67), finding that loss of WT1 does indeed interfere with the ability of these cells to tolerate changes in either the expression or function of STIM1, STIM2 or Orai1.

In an effort to examine bona-fide Wilms Tumor cells, we recently obtained a series of human Wilms Tumor explants maintained subcutaneously in SCID mice from Dr. Peter Houghton (Nationwide Hospital; Ohio) (90). We have now examined SOCe in 9 of these tumor explants, of which 8 were WT1⁺ and one was WT1-null. Consistent with our expectations, the amount of SOCe was many-fold higher in the WT1-null Wilms Tumor explant (unpublished observations). Although we have yet to fully establish the therapeutic implications of these observations, it is interesting to note that dysregulated expression of a STIM-independent Ca^{2+} channel (CaV2.3) is associated with Wilms Tumor relapse (91). Hence, current treatment regimens for Wilms tumor achieve 90% cure rates, but patients remain at high risk for tumor relapse at which point these tumors become much more difficult to treat (92). The extent to which SOCe function may also contribute to Wilms Tumor relapse has not been established, however, it is interesting that links between EGR1 expression on responsiveness to chemotherapy have been investigated (93). Interestingly, EGR1 expression correlated well with a robust response to chemotherapy, while decreased EGR1 levels were found in tumors with a limited response therapy (93). However, based on our examination of our panel of Wilms Tumors, all of the tumors exhibiting strong WT1 expression had little or no STIM1 expression or SOCe irrespective EGR1, which was highly variable (unpublished observations). This is presumably because, as shown in our recent paper (59), WT1 could outcompete EGR1 for binding to the STIM1 promoter. Therefore, as enticing as the possibility may be, we consider it unlikely that the relationship between Wilms Tumor relapse and EGR1 is due to differences in SOCe.

3.2. WT1, EGR1 and STIM1 in breast cancer

Breast cancer is the most common types of solid tumors, occurring in greater than 1 in 5 women. One of its defining features is a progression from estrogen receptor-positive (ER⁺) to ER⁻ tumor cells, with the loss of ER expression strongly correlating with poor outcomes. Based on numerous recent investigations, it is now clear that this shift to estrogen-independence includes numerous changes in gene expression patterns, including WT1, EGR1 and, perhaps, members of the STIM and Orai family. Hence, in ER⁺ breast cancer, loss of WT1 expression is required for dysregulated cell proliferation (83, 94); in this disease, WT1 functions as a tumor suppressor via interactions with ER- α leading to inhibition of insulin growth factor receptor expression (95). Since WT1 inhibits STIM1 expression, ER⁺ breast cancer cells would be predicted to have increased STIM1 expression, although the accuracy of this prediction has not been established. However, it has recently been shown that, unlike either normal epithelial or ER⁻ breast cancer cells, the channels mediating SOCe in this subclass of cells are predominantly Orai3 and not Orai1 (96). Hence,

the pathways regulating both STIM and Orai expression in this class of breast cancer cells exhibit significant novel features with potentially important implications.

In ER⁻ breast cancer, the effect of WT1 expression seems to shift from growth inhibitory to growth promoting; not only is WT1 upregulated (97), but this upregulation is correlated with poor prognosis (84). Further, introduction of WT1 antisense oligos results in growth inhibition (82). Considered collectively with reports of genetic deletion of EGR1 associated with ER⁻ breast cancer cells (98), we would predict these cells to exhibit significant loss of STIM1 expression. However, that does not appear to be the case. To the contrary, STIM1 and Orai1 are required for the migration and metastasis of the highly aggressive ER⁻ breast cancer cells, as demonstrated using both *in vitro* and *in vivo* models (99). Precisely how this class of tumor cell escapes WT1-mediated inhibition of STIM1 expression is unclear, however, it is interesting to note that the relative patterns of WT1 splice variant expression has been shown to shift such that exon 5 and KTS inserts are less efficiently spliced out of WT1 in these cells (83); an important consideration since only the shortest form of WT1 inhibits STIM1 expression (59). Nevertheless, we also recognize that induction of STIM1 expression in ER⁻ breast cancer cells may also be under the control of other EGR family members and/or as yet to be identified transcription factors.

In accordance with the notion that SOCe plays a pivotal role in breast cancer tumorigenesis, Yang and colleagues have shown that SOCe is crucial for the migration and metastasis of a highly aggressive breast cancer cell line *in vitro* and *in vivo* (99). Furthermore, the authors established that SOCe signaling regulates focal adhesion turnover and thus, by blocking Ca²⁺ influx, cell adhesions mediated by the interaction of integrin proteins with the extracellular matrix are lost (99). Accordingly, this study underscores the great potential that targeting SOCe may have as a therapeutic target for the treatment of cancer.

3.3. WT1, EGR1 and Ca²⁺ signaling in Acute Myeloid Leukemia

Acute Myeloid Leukemia (AML) is a highly heterogeneous and devastating disease; most patients diagnosed with this disease die within 2 years (100). Interestingly, WT1 has been found to be upregulated in 73 to 93% of primary AML samples (78). This is, perhaps, not surprising given that AML is characterized by a developmental block during hematopoiesis; WT1 is strongly expressed in CD34⁺ progenitor cells but is normally lost as they differentiate into mature leukocytes. EGR1, by contrast, promotes terminal myeloid differentiation (101, 102) thereby functioning as a tumor suppressor, although this role is highly dependent on the transforming oncogene (74). A direct examination of the relationships between WT1, EGR1 and STIM1 expression and function in AML has not been performed. However, in a prior study performed collaboratively with Dr. Stuart Berger (University Health Network, Toronto, CA), we examined SOCe in several AML cell lines (103). Interestingly, consistent with what might be expected for cells expressing WT1, but not EGR1, only minimal SOCe was observed in 3 out of 5 AML cell lines examined. Although 3 out of 5 might seem to be a somewhat weak correlation, this improves based on the fact that, murine 32D leukemia cells (which we showed had high SOCe) have been confirmed to lack WT1 expression (104). Further, amongst the cell lines exhibiting low SOCe, not only do HL60 cells express WT1, but in response to vitamin D3-induced differentiation into monocytes leads SOCe recovers (105), WT1 expression is lost (106) and EGR1 is induced (107). Precisely how these differential Ca²⁺ signals impact development, progression or treatment of AML has not been established. However, inactivating WT1 mutations, observed in 10–12% of patients, are a negative prognostic indicator for AML (78). Further, all of the WT1⁺ cell lines and primary cell types examined in our prior study could be virtually eliminated (~99.99% loss of clonogenicity) by the SOCe inhibitor econazole at concentrations that did not interfere with bone marrow reconstitution (103). Given our new insight into the identities of the molecular mediators of SOCe and the roles

of WT1 and EGR1 as regulators of STIM1 expression, we are currently in the process of revisiting these studies to assess the contribution of Ca^{2+} signals towards the progression and/or treatment of this disease.

3.4. EGR1 and STIM1 expression in Glioblastoma

Virtually all brain cancers result from transformation of glial cells, the non-neuronal support cell found throughout the central nervous system. Glioblastoma multiforme is the most common and aggressive type of glioma in humans, accounting for 52% of all primary brain tumor cases and 20% of all intracranial tumors. Due to its aggressive nature and resistance to most conventional therapeutic strategies, the median survival time is 18 months. As such, there is a great need for new insight into both glioblastoma biology and alternative therapeutic strategies. Over the last 15 years, there have been a number of tantalizing clues that both EGR1 and Ca^{2+} homeostasis may represent novel and untapped targets in this cell type. Thus, glioblastoma cells are highly dependent on SOCs for extracellular Ca^{2+} influx (108), which is significantly enhanced compared with normal astrocytes (109). Further, this Ca^{2+} influx has been shown to affect cell cycle progression in this model (110, 111). In addition, Ca^{2+} -dependent activation of CaM Kinase III leads to high levels of autophagy, which enhanced resistance to nutrient deprivation-induced apoptosis (112). However, more recently, it has been shown glioblastoma cell survival is enhanced by decreasing ER Ca^{2+} release via Akt-mediated inhibition of InsP₃R function via phosphorylation (113). Further, glioblastoma cells exhibit relatively high susceptibility to induction of ER stress via ER Ca^{2+} release (114). Considered collectively, these observations suggest that while Ca^{2+} entry supports the survival and growth of glioblastoma, they are highly sensitive to differences in ER Ca^{2+} levels.

Recent analyses of the gene expression profiles of primary glioblastoma and normal brain tissue, it was found that the levels of both STIM1 (115) and STIM2 (116) were significantly higher in glioblastoma. However, the extent to which this upregulation of STIM1 and STIM2 is related to EGR1 expression is not known. Indeed, exactly what happens to EGR1 in glioblastoma remains somewhat controversial. Hence, hyperactivity of EGR1 due to upregulation of the EGF and PDGF α receptors has been reported in several glioblastoma cell lines (117), potentially accounting for upregulated STIM expression (115, 116) and Ca^{2+} influx (108). Further, this EGR1 upregulation was associated with enhanced cell motility and metastasis through transactivation of the fibronectin gene (117). However, EGR1 was originally identified as a tumor suppressor in glioblastoma, where it was thought to be both downregulated and growth inhibitory (118). This principle was further supported with the report that NMDA-mediated induction of EGR1 expression was abrogated in primary glioblastoma, an abrogation that was associated with decreased patient survival (119). Like most tumor types, not only are there multiple initiating events for glioblastoma, but the disease has several stages of progression during which signaling pathways become increasingly dysregulated. Towards this end, there are also instances where WT1 expression is increased in glioblastoma; a characteristic which increases tumorigenicity (120–124) and decreases the radiosensitivity of the tumor *in vitro* and *in vivo* (121). Hence, determining the precise characteristics of the cells in which EGR1 performs these mutually opposing roles is undoubtedly the critical first step in understanding how this gene contributes to glioblastoma cell biology (120–123).

3.5. WT1/EGR1-mediated control of SOCs in prostate cancer

Prostate cancer is one of the leading threats to men's health. Similar to breast cancer, in its early stages, it is highly dependent on steroid production for growth, although, in this case androgens rather than estrogens are the steroids responsible (125). Consequently, most therapies currently in use target either the androgen receptor or androgen production. As

such, it is intriguing that androgen receptor expression can be downregulated due to increases in intracellular Ca^{2+} concentration (126). Even more intriguing, a series of studies performed in LNCaP cells reveal that when they are transformed to an androgen-independent phenotype (via Bcl2 overexpression, androgen withdrawal or pharmacological upregulation of cAMP), they exhibit decreases in both ER Ca^{2+} content and SOCe (127, 128). Given our recent findings (59), it is tempting to speculate that this change in Ca^{2+} homeostasis may reflect differences in the expression of WT1 and EGR1. Indeed, there is ample evidence of dysregulation of WT1 and EGR1 in prostate cancer. Thus, both the expression and function of EGR1 have been shown to be greatly enhanced in prostate cancer (129–131). Further, EGR1-KO prostate cells exhibit impaired tumorigenesis (76), implying that upregulation of EGR1 serves as a crucial promoter in the initiation of this disease. On the other hand, examination of the patterns of WT1 and EGR1 expression in several prostate cancer cell lines revealed the exact opposite profile; elevated WT1 expression coinciding with low EGR1 expression (80). While the extent to which the expression patterns in these cell lines reflects WT1 and EGR1 expression in vivo is less than clear, it is conceivable that this high WT1, low EGR1 expression pattern supports an androgen-independent phenotype via direct or indirect mechanisms.

The intense interest in the role of Ca^{2+} in the development of prostate cancer led to a number of attempts to target Ca^{2+} homeostasis as a potential treatment for this disease, particularly the currently untreatable androgen-independent variants. Indeed, it was shown over 15 years ago that androgen-independent prostate cancer cells can be induced to undergo apoptosis in the presence of the SERCA inhibitor thapsigargin (132). However, despite significant efforts to modify thapsigargin to selectively target prostate cancer cells (133, 134), the general toxicity of this compound has made it unsuitable as a therapeutic agent. By contrast, SOCe seems to be a far more viable target, since genetic mutations leading to loss of SOCe lead to relatively minor problems outside of Severe-Combined Immunodeficiency (SCID) (30, 135); temporary immune defects due to pharmacological inhibition of SOCe would be a highly acceptable trade-off if they were effective as a therapeutic for androgen-independent prostate cancer. While the pharmacological agents currently available that target SOCe exhibit questionable specificity, the identification of Orail as the pore forming unit of SOCe has undoubtedly sparked new efforts to design superior SOCe-targeting drugs. Should these efforts be successful, assessing their potential abilities to control this disease should be a high priority.

3.6. WT1/EGR1 and Ovarian Carcinoma

Ovarian carcinoma is the leading cause of death from gynecologic malignancies (136). Ovarian carcinoma (in general) occurs due to the need for remodeling of the epithelium after repeated menstrual cycles. Hence, every time an oocyte is released from the ovary, the epithelium has to be broken and then reformed. During postovulatory repair, lack of contact inhibition can cause ovarian epithelial cells to transform into mesenchymal cells, a process termed epithelial-mesenchyme transition (EMT) (137). EMT imparts an advantage to the postovulatory repair of the ovarian epithelium by altering the motility and proliferative response and allows for proper remodeling of the ovarian surface epithelium (138). However, mesenchymal cells are prone to uncontrolled growth and transformation into cancer cells.

Whereas little has been documented to demonstrate a relationship between EGR1 and ovarian carcinoma, WT1 is known to regulate the mesenchymal/epithelial balance during development and several lines of evidence point to a role of WT1 in both EMT and mesenchyme to epithelial transition (MET) (139–143). Thus, it is interesting to speculate that aberrant WT1 expression in ovarian tumors causes cells to retain a mesenchymal phenotype in early ovarian tumorigenesis. This concept is supported by the fact that WT1

expression plays an important role in the progression of ovarian tumors and indicates a poorer prognosis of ovarian carcinoma (144–148). Still unknown is whether or not suppression of STIM1 expression and SOCe by WT1 has any impact on the epithelial/mesenchymal balance. However, it should be noted that mesenchyme to epithelial transitions are accompanied by profound changes in the expression and activity of plasma membrane chloride and potassium ion channels (149). Considered collectively with our observations regarding WT1-mediated SOCe inhibition (59), it seems reasonable to speculate that dysregulated Ca^{2+} homeostasis may also be an important stabilizing characteristic of mesenchymal cells. Indeed, E-cadherin, an epithelial cell adhesion molecule which can be regulated by Ca^{2+} -dependent Ras activity (150, 151), is frequently absent or mutated in ovarian carcinoma (152) which promotes invasion and metastasis (153). Therefore, inhibition of Ca^{2+} entry by WT1 would tend to inhibit E-cadherin-mediated cell-cell adhesion, ultimately supporting a similar dysregulated metastatic phenotype.

4. Summary and perspective

Aberrant Ca^{2+} signaling in cancer cells has been under investigation for several decades, yet there is still a great deal of confusion about how Ca^{2+} contributes to cancer cell biology. Cancer is predominantly a disease of disordered balance between proliferation, differentiation and apoptosis; calcium signals can contribute to all 3 outcomes, however, precisely how depends on which other changes related to these outcomes coincide with dysregulated Ca^{2+} homeostasis. For example, increased Ca^{2+} influx could stimulate Ca^{2+} -dependent proliferative and/or migratory pathways (eg. breast cancer, glioblastoma, prostate cancer), yet suppression of SOCe can inhibit differentiation, thereby trapping cells in a pluripotent, proliferative state (eg. Wilms tumor, AML, ovarian cancer). Determining how and why Ca^{2+} signals become dysregulated in specific classes of cancer cells is critical to designing therapeutic strategies targeting Ca^{2+} signals. Our observation that WT1 and EGR1 regulate STIM1 expression (59) has provided an important new tool to address this problem. Hence, as a developmentally regulated gene, WT1 is aberrantly expressed in a wide variety of cancer cells. Similarly, the sheer variety of the signaling pathways in control of EGR1 expression (Fig. 2) makes it a very prominent oncogene/tumor suppressor. As such, WT1 and EGR1 have significant potential as biomarkers, offering crucial insight into how Ca^{2+} signaling is changed in specific cell types, potentially leading to novel new therapeutic strategies targeting loss of control over Ca^{2+} homeostasis in cancer cells.

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References

1. Boynton AL, Whitfield JF, Isaacs RJ, Morton HJ. Control of 3T3 cell proliferation by calcium. In *Vitro*. 1974; 10:12–17. [PubMed: 4471173]
2. Whitfield JF, Boynton AL, MacManus JP, Sikorska M, Tsang BK. The regulation of cell proliferation by calcium and cyclic AMP. *Mol Cell Biochem*. 1979; 27(3):155–179. [PubMed: 229407]
3. Berridge MJ, Irvine RF. Inositol trisphosphate, a novel second messenger in cellular signal transduction. *Nature*. 1984; 312(5992):315–321. [PubMed: 6095092]
4. Bridges K, Levenson R, Housman D, Cantley L. Calcium regulates the commitment of murine erythroleukemia cells to terminal erythroid differentiation. *J Cell Biol*. 1981; 90(2):542–544. [PubMed: 6793600]
5. Holliday J, Adams RJ, Sejnowski TJ, Spitzer NC. Calcium-induced release of calcium regulates differentiation of cultured spinal neurons. *Neuron*. 1991; 7(5):787–796. [PubMed: 1742025]

6. Orrenius S, Zhivotovsky B, Nicotera P. Regulation of cell death: the calcium-apoptosis link. *Nat Rev Mol Cell Biol.* 2003; 4(7):552–565. [PubMed: 12838338]
7. Boynton AL, Whitfield JF. Different calcium requirements for proliferation of conditionally and unconditionally tumorigenic mouse cells. *Proc Natl Acad Sci U S A.* 1976; 73(5):1651–1654. [PubMed: 1064038]
8. Boynton AL, Whitfield JF. Calcium requirements for the proliferation of cells infected with a temperature-sensitive mutant of Rous sarcoma virus. *Cancer Res.* 1978; 38(5):1237–1240. [PubMed: 205359]
9. Whitfield JF. Calcium signals and cancer. *Crit Rev Oncog.* 1992; 3(1–2):55–90. [PubMed: 1550862]
10. Adams JM, Cory S. The Bcl-2 apoptotic switch in cancer development and therapy. *Oncogene.* 2007; 26(9):1324–1337. 10.1038/sj.onc.1210220. [PubMed: 17322918]
11. Frenzel A, Grespi F, Chmielewski W, Villunger A. Bcl2 family proteins in carcinogenesis and the treatment of cancer. *Apoptosis.* 2009; 14(4):584–596. [PubMed: 19156528]
12. Lomonosova E, Chinnadurai G. BH3-only proteins in apoptosis and beyond: an overview. *Oncogene.* 2008; 27(Suppl 1):S2–S19. 10.1038/onc.2009.39. [PubMed: 19641503]
13. Eckenrode EF, Yang J, Velmurugan GV, Foskett JK, White C. Apoptosis protection by Mcl-1 and Bcl-2 modulation of inositol 1,4,5-trisphosphate receptor-dependent Ca^{2+} signaling. *J Biol Chem.* 2010; 285(18):13678–13684. 10.1074/jbc.M109.096040. [PubMed: 20189983]
14. Li C, Wang X, Vais H, Thompson CB, Foskett JK, White C. Apoptosis regulation by Bcl-x(L) modulation of mammalian inositol 1,4,5-trisphosphate receptor channel isoform gating. *Proc Natl Acad Sci U S A.* 2007; 104(30):12565–12570. 10.1073/pnas.0702489104. [PubMed: 17636122]
15. White C, Li C, Yang J, Petrenko NB, Madesh M, Thompson CB, Foskett JK. The endoplasmic reticulum gateway to apoptosis by Bcl-X(L) modulation of the InsP_3R . *Nat Cell Biol.* 2005; 7(10):1021–1028. 10.1038/ncb1302. [PubMed: 16179951]
16. Pinton P, Ferrari D, Magalhaes P, Schulze-Osthoff K, Di Virgilio F, Pozzan T, Rizzuto R. Reduced loading of intracellular Ca^{2+} stores and downregulation of capacitative Ca^{2+} influx in Bcl-2-overexpressing cells. *J Cell Biol.* 2000; 148(5):857–862. [PubMed: 10704437]
17. Foyouzi-Youssefi R, Arnaudeau S, Borner C, Kelley WL, Tschopp J, Lew DP, Demarex N, Krause KH. Bcl-2 decreases the free Ca^{2+} concentration within the endoplasmic reticulum. *Proc Natl Acad Sci U S A.* 2000; 97(11):5723–5728. [PubMed: 10823933]
18. Rong YP, Bultynck G, Aromolaran AS, Zhong F, Parys JB, De Smedt H, Mignery GA, Roderick HL, Bootman MD, Distelhorst CW. The BH4 domain of Bcl-2 inhibits ER calcium release and apoptosis by binding the regulatory and coupling domain of the IP_3 receptor. *Proc Natl Acad Sci U S A.* 2009; 106(34):14397–14402. [PubMed: 19706527]
19. Rong YP, Aromolaran AS, Bultynck G, Zhong F, Li X, McColl K, Matsuyama S, Herlitze S, Roderick HL, Bootman MD, Mignery GA, Parys JB, De Smedt H, Distelhorst CW. Targeting Bcl-2- IP_3 receptor interaction to reverse Bcl-2's inhibition of apoptotic calcium signals. *Mol Cell.* 2008; 31(2):255–265. 10.1016/j.molcel.2008.06.014. [PubMed: 18657507]
20. Oakes SA, Scorrano L, Opferman JT, Bassik MC, Nishino M, Pozzan T, Korsmeyer SJ. Proapoptotic BAX and BAK regulate the type 1 inositol trisphosphate receptor and calcium leak from the endoplasmic reticulum. *Proc Natl Acad Sci U S A.* 2005; 102(1):105–110. [PubMed: 15613488]
21. Venkatachalam K, Montell C. TRP channels. *Annu Rev Biochem.* 2007; 76:387–417. [PubMed: 17579562]
22. Duncan LM, Deeds J, Hunter J, Shao J, Holmgren LM, Woolf EA, Tepper RI, Shyjan AW. Down-regulation of the novel gene melastatin correlates with potential for melanoma metastasis. *Cancer Res.* 1998; 58(7):1515–1520. [PubMed: 9537257]
23. Tsavaler L, Shapero MH, Morkowski S, Laus R. Trp-p8, a novel prostate-specific gene, is up-regulated in prostate cancer and other malignancies and shares high homology with transient receptor potential calcium channel proteins. *Cancer Res.* 2001; 61(9):3760–3769. [PubMed: 11325849]

24. Peng JB, Zhuang L, Berger UV, Adam RM, Williams BJ, Brown EM, Hediger MA, Freeman MR. CaT1 expression correlates with tumor grade in prostate cancer. *Biochem Biophys Res Commun*. 2001; 282(3):729–734. [PubMed: 11401523]
25. Lehen'kyi V, Flourakis M, Skryma R, Prevarskaya N. TRPV6 channel controls prostate cancer cell proliferation via Ca(2+)/NFAT-dependent pathways. *Oncogene*. 2007; 26(52):7380–7385. [PubMed: 17533368]
26. El Boustany C, Bidaux G, Enfissi A, Delcourt P, Prevarskaya N, Capiod T. Capacitative calcium entry and transient receptor potential canonical 6 expression control human hepatoma cell proliferation. *Hepatology*. 2008; 47(6):2068–2077. [PubMed: 18506892]
27. Putney JW Jr. A model for receptor-regulated calcium entry. *Cell Calcium*. 1986; 7(1):1–12. [PubMed: 2420465]
28. Liou J, Kim ML, Heo WD, Jones JT, Myers JW, Ferrell JE Jr, Meyer T. STIM is a Ca²⁺ sensor essential for Ca²⁺-store-depletion-triggered Ca²⁺ influx. *Curr Biol*. 2005; 15(13):1235–1241. [PubMed: 16005298]
29. Roos J, Digregorio PJ, Yeromin AV, Ohlsen K, Lioudyno M, Zhang S, Safrina O, Kozak JA, Wagner SL, Cahalan MD, Velicelebi G, Stauderman KA. STIM1, an essential and conserved component of store-operated Ca²⁺ channel function. *J Cell Biol*. 2005; 169(3):435–445. [PubMed: 15866891]
30. Feske S, Gwack Y, Prakriya M, Srikanth S, Puppel SH, Tanasa B, Hogan PG, Lewis RS, Daly M, Rao A. A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. *Nature*. 2006; 441(7090):179–185. [PubMed: 16582901]
31. Vig M, Peinelt C, Beck A, Koomoa DL, Rabah D, Koblan-Huberson M, Kraft S, Turner H, Fleig A, Penner R, Kinet JP. CRACM1 is a plasma membrane protein essential for store-operated Ca²⁺ entry. *Science*. 2006; 312(5777):1220–1223. [PubMed: 16645049]
32. Zhang SL, Yeromin AV, Zhang XH, Yu Y, Safrina O, Penna A, Roos J, Stauderman KA, Cahalan MD. 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*. 2006; 103(24):9357–9362. [PubMed: 16751269]
33. Hewavitharana T, Deng X, Soboloff J, Gill DL. Role of STIM and Orai proteins in the store-operated calcium signaling pathway. *Cell Calcium*. 2007; 42(2):173–182. [PubMed: 17602740]
34. Luik RM, Lewis RS. New insights into the molecular mechanisms of store-operated Ca²⁺ signaling in T cells. *Trends Mol Med*. 2007; 13(3):103–107. [PubMed: 17267286]
35. Soboloff J, Spassova MA, Tang XD, Hewavitharana T, Xu W, Gill DL. Orai1 and STIM Reconstitute Store-operated Calcium Channel Function. *J Biol Chem*. 2006; 281(30):20661–20665. [PubMed: 16766533]
36. Feske S. Calcium signalling in lymphocyte activation and disease. *Nat Rev Immunol*. 2007; 7(9):690–702. [PubMed: 17703229]
37. Hogan PG, Rao A. Dissecting ICRCAC, a store-operated calcium current. *Trends Biochem Sci*. 2007; 32(5):235–245. [PubMed: 17434311]
38. Deng X, Wang Y, Zhou Y, Soboloff J, Gill DL. STIM and Orai: dynamic intermembrane coupling to control cellular calcium signals. *J Biol Chem*. 2009; 284(34):22501–22505. [PubMed: 19473984]
39. Williams RT, Manji SS, Parker NJ, Hancock MS, Van Stekelenburg L, Eid JP, Senior PV, Kazenwadel JS, Shandala T, Saint R, Smith PJ, Dziadek MA. Identification and characterization of the STIM (stromal interaction molecule) gene family: coding for a novel class of transmembrane proteins. *Biochem J*. 2001; 357(Pt 3):673–685. [PubMed: 11463338]
40. Stathopulos PB, Zheng L, Li GY, Plevin MJ, Ikura M. Structural and mechanistic insights into STIM1-mediated initiation of store-operated calcium entry. *Cell*. 2008; 135(1):110–122. [PubMed: 18854159]
41. Stathopulos PB, Li GY, Plevin MJ, Ames JB, Ikura M. Stored Ca²⁺ depletion-induced oligomerization of stromal interaction molecule 1 (STIM1) via the EF-SAM region: An initiation mechanism for capacitive Ca²⁺ entry. *J Biol Chem*. 2006; 281(47):35855–35862. [PubMed: 17020874]

42. Stathopoulos PB, Zheng L, Ikura M. Stromal Interaction Molecule (STIM) 1 and STIM2 Calcium Sensing Regions Exhibit Distinct Unfolding and Oligomerization Kinetics. *J Biol Chem.* 2009; 284(2):728–732. [PubMed: 19019825]
43. Hewavitharana T, Deng X, Wang Y, Ritchie MF, Girish GV, Soboloff J, Gill DL. Location and function of STIM1 in the activation of Ca^{2+} entry signals. *J Biol Chem.* 2008; 283(38):26252–26262. [PubMed: 18635545]
44. Luik RM, Wang B, Prakriya M, Wu MM, Lewis RS. Oligomerization of STIM1 couples ER calcium depletion to CRAC channel activation. *Nature.* 2008; 454(7203):538–542. [PubMed: 18596693]
45. Wu MM, Buchanan J, Luik RM, Lewis RS. Ca^{2+} store depletion causes STIM1 to accumulate in ER regions closely associated with the plasma membrane. *J Cell Biol.* 2006; 174(6):803–813. [PubMed: 16966422]
46. Luik RM, Wu MM, Buchanan J, Lewis RS. The elementary unit of store-operated Ca^{2+} entry: local activation of CRAC channels by STIM1 at ER-plasma membrane junctions. *J Cell Biol.* 2006; 174(6):815–825. [PubMed: 16966423]
47. Wang Y, Deng X, Zhou Y, Hendron E, Mancarella S, Ritchie MF, Tang XD, Baba Y, Kurosaki T, Mori Y, Soboloff J, Gill DL. STIM protein coupling in the activation of Orai channels. *Proc Natl Acad Sci U S A.* 2009; 106(18):7391–7396. [PubMed: 19376967]
48. Muik M, Fahrner M, Derler I, Schindl R, Bergsmann J, Frischauf I, Groschner K, Romanin C. A Cytosolic Homomerization and a Modulatory Domain within STIM1 C Terminus Determine Coupling to ORAI1 Channels. *J Biol Chem.* 2009; 284(13):8421–8426. [PubMed: 19189966]
49. Park CY, Hoover PJ, Mullins FM, Bachhawat P, Covington ED, Raunser S, Walz T, Garcia KC, Dolmetsch RE, Lewis RS. STIM1 clusters and activates CRAC channels via direct binding of a cytosolic domain to Orai1. *Cell.* 2009; 136(5):876–890. [PubMed: 19249086]
50. Yuan JP, Zeng W, Dorwart MR, Choi YJ, Worley PF, Muallem S. SOAR and the polybasic STIM1 domains gate and regulate Orai channels. *Nat Cell Biol.* 2009; 11(3):337–343. [PubMed: 19182790]
51. Mercer JC, Dehaven WI, Smyth JT, Wedel B, Boyles RR, Bird GS, Putney JW Jr. Large store-operated calcium selective currents due to co-expression of Orai1 or Orai2 with the intracellular calcium sensor, Stim1. *J Biol Chem.* 2006; 281(34):24979–24990. [PubMed: 16807233]
52. Peinelt C, Vig M, Koomoa DL, Beck A, Nadler MJ, Koblan-Huberson M, Lis A, Fleig A, Penner R, Kinet JP. Amplification of CRAC current by STIM1 and CRACM1 (Orai1). *Nat Cell Biol.* 2006; 8(7):771–773. [PubMed: 16733527]
53. Brandman O, Liou J, Park WS, Meyer T. STIM2 is a feedback regulator that stabilizes basal cytosolic and endoplasmic reticulum Ca^{2+} levels. *Cell.* 2007; 131(7):1327–1339. [PubMed: 18160041]
54. Parvez S, Beck A, Peinelt C, Soboloff J, Lis A, Monteilh-Zoller M, Gill DL, Fleig A, Penner R. STIM2 protein mediates distinct store-dependent and store-independent modes of CRAC channel activation. *Faseb J.* 2008; 22(3):752–761. [PubMed: 17905723]
55. Soboloff J, Spassova MA, Hewavitharana T, He LP, Xu W, Johnstone LS, Dziadek MA, Gill DL. STIM2 Is an Inhibitor of STIM1-Mediated Store-Operated Ca^{2+} Entry. *Curr Biol.* 2006; 16(14):1465–1470. [PubMed: 16860747]
56. Zhou Y, Mancarella S, Wang Y, Yue C, Ritchie M, Gill DL, Soboloff J. The Short N-terminal Domains of STIM1 and STIM2 Control the Activation Kinetics of Orai1 Channels. *J Biol Chem.* 2009; 284(29):19164–19168. [PubMed: 19487696]
57. DeHaven WI, Smyth JT, Boyles RR, Putney JW Jr. Calcium inhibition and calcium potentiation of Orai1, Orai2, and Orai3 calcium release-activated calcium channels. *J Biol Chem.* 2007; 282(24):17548–17556. [PubMed: 17452328]
58. Lis A, Peinelt C, Beck A, Parvez S, Monteilh-Zoller M, Fleig A, Penner R. CRACM1, CRACM2, and CRACM3 are store-operated Ca^{2+} channels with distinct functional properties. *Curr Biol.* 2007; 17(9):794–800. [PubMed: 17442569]
59. Ritchie MF, Yue C, Zhou Y, Houghton PJ, Soboloff J. Wilms Tumor Suppressor 1 (WT1) and Early Growth Response 1 (EGR1) Are Regulators of STIM1 Expression. *J Biol Chem.* 2010; 285(14):10591–10596. [PubMed: 20123987]

60. Beckmann AM, Matsumoto I, Wilce PA. AP-1 and Egr DNA-binding activities are increased in rat brain during ethanol withdrawal. *J Neurochem.* 1997; 69(1):306–314. [PubMed: 9202324]
61. Gashler A, Sukhatme VP. Early growth response protein 1 (Egr-1): prototype of a zinc-finger family of transcription factors. *Prog Nucleic Acid Res Mol Biol.* 1995; 50:191–224. [PubMed: 7754034]
62. Wang C, Dostanic S, Servant N, Chalifour LE. Egr-1 negatively regulates expression of the sodium-calcium exchanger-1 in cardiomyocytes in vitro and in vivo. *Cardiovasc Res.* 2005; 65(1): 187–194. [PubMed: 15621046]
63. Kasneci A, Kemeny-Suss NM, Komarova SV, Chalifour LE. Egr-1 negatively regulates calsequestrin expression and calcium dynamics in ventricular cells. *Cardiovasc Res.* 2009; 81(4): 695–702. [PubMed: 19103607]
64. Brady M, Koban MU, Dellow KA, Yacoub M, Boheler KR, Fuller SJ. Sp1 and Sp3 transcription factors are required for trans-activation of the human SERCA2 promoter in cardiomyocytes. *Cardiovasc Res.* 2003; 60(2):347–354. [PubMed: 14613864]
65. Hara S, Arai M, Tomaru K, Doi H, Koitabashi N, Iso T, Watanabe A, Tanaka T, Maeno T, Suga T, Yokoyama T, Kurabayashi M. Prostaglandin F2alpha inhibits SERCA2 gene transcription through an induction of Egr-1 in cultured neonatal rat cardiac myocytes. *Int Heart J.* 2008; 49(3):329–342. [PubMed: 18612190]
66. Arai M, Yoguchi A, Takizawa T, Yokoyama T, Kanda T, Kurabayashi M, Nagai R. Mechanism of doxorubicin-induced inhibition of sarcoplasmic reticulum Ca(2+)-ATPase gene transcription. *Circ Res.* 2000; 86(1):8–14. [PubMed: 10625299]
67. Zhou Y, Ritchie MF, Yue C, Madesh M, Soboloff J. Targeting Store-operated Ca²⁺ Entry to Induce Cell Death depends on WT1-Regulated SERCA2 expression. *Cell Death Differ.* 2010 in revision.
68. Scharnhorst V, Menke AL, Attema J, Haneveld JK, Riteco N, van Steenbrugge GJ, van der Eb AJ, Jochemsen AG. EGR-1 enhances tumor growth and modulates the effect of the Wilms' tumor 1 gene products on tumorigenicity. *Oncogene.* 2000; 19(6):791–800. [PubMed: 10698497]
69. Bae SK, Bae MH, Ahn MY, Son MJ, Lee YM, Bae MK, Lee OH, Park BC, Kim KW. Egr-1 mediates transcriptional activation of IGF-II gene in response to hypoxia. *Cancer Res.* 1999; 59(23):5989–5994. [PubMed: 10606246]
70. Hamilton TB, Borel F, Romaniuk PJ. Comparison of the DNA binding characteristics of the related zinc finger proteins WT1 and EGR1. *Biochemistry.* 1998; 37(7):2051–2058. [PubMed: 9485332]
71. Morrison AA, Viney RL, Ladomery MR. The post-transcriptional roles of WT1, a multifunctional zinc-finger protein. *Biochim Biophys Acta.* 2008; 1785(1):55–62. [PubMed: 17980713]
72. Nurmammedov E, Yengo RK, Uysal H, Karlsson R, Thunnissen MM. New insights into DNA-binding behavior of Wilms tumor protein (WT1)--a dual study. *Biophys Chem.* 2009; 145(2–3): 116–125. [PubMed: 19853363]
73. Richard DJ, Schumacher V, Royer-Pokora B, Roberts SG. Par4 is a coactivator for a splice isoform-specific transcriptional activation domain in WT1. *Genes Dev.* 2001; 15(3):328–339. [PubMed: 11159913]
74. Gibbs JD, Liebermann DA, Hoffman B. Leukemia suppressor function of Egr-1 is dependent on transforming oncogene. *Leukemia.* 2008; 22(10):1909–1916. [PubMed: 18650844]
75. Redmond KL, Crawford NT, Farmer H, D'Costa ZC, O'Brien GJ, Buckley NE, Kennedy RD, Johnston PG, Harkin DP, Mullan PB. T-box 2 represses NDRG1 through an EGR1-dependent mechanism to drive the proliferation of breast cancer cells. *Oncogene.* 2010; 29(22):3252–3262. [PubMed: 20348948]
76. Abdulkadir SA, Qu Z, Garabedian E, Song SK, Peters TJ, Svaren J, Carbone JM, Naughton CK, Catalona WJ, Ackerman JJ, Gordon JI, Humphrey PA, Milbrandt J. Impaired prostate tumorigenesis in Egr1-deficient mice. *Nat Med.* 2001; 7(1):101–107. [PubMed: 11135623]
77. Rivera MN, Haber DA. Wilms' tumour: connecting tumorigenesis and organ development in the kidney. *Nat Rev Cancer.* 2005; 5(9):699–712. [PubMed: 16110318]
78. Owen C, Fitzgibbon J, Paschka P. The clinical relevance of Wilms Tumour 1 (WT1) gene mutations in acute leukaemia. *Hematol Oncol.* 2010; 28(1):13–19. [PubMed: 20013787]

79. Yang L, Han Y, Suarez Saiz F, Minden MD. A tumor suppressor and oncogene: the WT1 story. *Leukemia*. 2007; 21(5):868–876. [PubMed: 17361230]
80. Gregg JL, Brown KE, Mintz EM, Piontkivska H, Fraizer GC. Analysis of gene expression in prostate cancer epithelial and interstitial stromal cells using laser capture microdissection. *BMC Cancer*. 2010; 10:165. [PubMed: 20426842]
81. Baron V, Adamson ED, Calogero A, Ragona G, Mercola D. The transcription factor Egr1 is a direct regulator of multiple tumor suppressors including TGFbeta1, PTEN, p53, and fibronectin. *Cancer Gene Ther*. 2006; 13(2):115–124. [PubMed: 16138117]
82. Zapata-Benavides P, Tuna M, Lopez-Berestein G, Tari AM. Downregulation of Wilms' tumor 1 protein inhibits breast cancer proliferation. *Biochem Biophys Res Commun*. 2002; 295(4):784–790. [PubMed: 12127961]
83. Silberstein GB, Van Horn K, Strickland P, Roberts CT Jr, Daniel CW. Altered expression of the WT1 wilms tumor suppressor gene in human breast cancer. *Proc Natl Acad Sci U S A*. 1997; 94(15):8132–8137. [PubMed: 9223327]
84. Miyoshi Y, Ando A, Egawa C, Taguchi T, Tamaki Y, Tamaki H, Sugiyama H, Noguchi S. High expression of Wilms' tumor suppressor gene predicts poor prognosis in breast cancer patients. *Clin Cancer Res*. 2002; 8(5):1167–1171. [PubMed: 12006533]
85. Kaufmann K, Thiel G. Epidermal growth factor and platelet-derived growth factor induce expression of Egr-1, a zinc finger transcription factor, in human malignant glioma cells. *J Neurol Sci*. 2001; 189(1–2):83–91. [PubMed: 11535237]
86. Takahashi M, Yang XJ, Lavery TT, Furge KA, Williams BO, Tretiakova M, Montag A, Vogelzang NJ, Re GG, Garvin AJ, Soderhall S, Kagawa S, Hazel-Martin D, Nordenskjold A, Teh BT. Gene expression profiling of favorable histology Wilms tumors and its correlation with clinical features. *Cancer Res*. 2002; 62(22):6598–6605. [PubMed: 12438255]
87. Garvin AJ, Re GG, Tarnowski BI, Hazen-Martin DJ, Sens DA. The G401 cell line, utilized for studies of chromosomal changes in Wilms' tumor, is derived from a rhabdoid tumor of the kidney. *Am J Pathol*. 1993; 142(2):375–380. [PubMed: 8382007]
88. Sabbioni S, Barbanti-Brodano G, Croce CM, Negrini M. GOK: a gene at 11p15 involved in rhabdomyosarcoma and rhabdoid tumor development. *Cancer Res*. 1997; 57(20):4493–4497. [PubMed: 9377559]
89. Sabbioni S, Veronese A, Trubia M, Taramelli R, Barbanti-Brodano G, Croce CM, Negrini M. Exon structure and promoter identification of STIM1 (alias GOK), a human gene causing growth arrest of the human tumor cell lines G401 and RD. *Cytogenet Cell Genet*. 1999; 86(3–4):214–218. [PubMed: 10575208]
90. Houghton PJ, Morton CL, Tucker C, Payne D, Favours E, Cole C, Gorlick R, Kolb EA, Zhang W, Lock R, Carol H, Tajbakhsh M, Reynolds CP, Maris JM, Courtright J, Keir ST, Friedman HS, Stopford C, Zeidner J, Wu J, Liu T, Billups CA, Khan J, Ansher S, Zhang J, Smith MA. The pediatric preclinical testing program: description of models and early testing results. *Pediatr Blood Cancer*. 2007; 49(7):928–940. [PubMed: 17066459]
91. Natrajan R, Little SE, Reis-Filho JS, Hing L, Messahel B, Grundy PE, Dome JS, Schneider T, Vujanic GM, Pritchard-Jones K, Jones C. Amplification and overexpression of CACNA1E correlates with relapse in favorable histology Wilms' tumors. *Clin Cancer Res*. 2006; 12(24):7284–7293. [PubMed: 17189400]
92. Pritchard-Jones K, Pritchard J. Success of clinical trials in childhood Wilms' tumour around the world. *Lancet*. 2004; 364(9444):1468–1470. [PubMed: 15500874]
93. Wittmann S, Wunder C, Zirn B, Furtwangler R, Wegert J, Graf N, Gessler M. New prognostic markers revealed by evaluation of genes correlated with clinical parameters in Wilms tumors. *Genes Chromosomes Cancer*. 2008; 47(5):386–395. [PubMed: 18260125]
94. Zhang TF, Yu SQ, Guan LS, Wang ZY. Inhibition of breast cancer cell growth by the Wilms' tumor suppressor WT1 is associated with a destabilization of beta-catenin. *Anticancer Res*. 2003; 23(5A):3575–3584. [PubMed: 14666652]
95. Reizner N, Maor S, Sarfstein R, Abramovitch S, Welshons WV, Curran EM, Lee AV, Werner H. The WT1 Wilms' tumor suppressor gene product interacts with estrogen receptor-alpha and

- regulates IGF-I receptor gene transcription in breast cancer cells. *J Mol Endocrinol.* 2005; 35(1): 135–144. [PubMed: 16087727]
96. Motiani RK, Abdullaev IF, Trebak M. A novel native store-operated calcium channel encoded by Orai3: selective requirement of Orai3 versus Orai1 in estrogen receptor-positive versus estrogen receptor-negative breast cancer cells. *J Biol Chem.* 2010; 285(25):19173–19183. [PubMed: 20395295]
 97. Loeb DM, Evron E, Patel CB, Sharma PM, Niranjana B, Buluwela L, Weitzman SA, Korz D, Sukumar S. Wilms' tumor suppressor gene (WT1) is expressed in primary breast tumors despite tumor-specific promoter methylation. *Cancer Res.* 2001; 61(3):921–925. [PubMed: 11221883]
 98. Ronski K, Sanders M, Burleson JA, Moyo V, Benn P, Fang M. Early growth response gene 1 (EGR1) is deleted in estrogen receptor-negative human breast carcinoma. *Cancer.* 2005; 104(5): 925–930. [PubMed: 15999367]
 99. Yang S, Zhang JJ, Huang XY. Orai1 and STIM1 are critical for breast tumor cell migration and metastasis. *Cancer Cell.* 2009; 15(2):124–134. [PubMed: 19185847]
 100. Walter RB, Appelbaum FR, Tallman MS, Weiss NS, Larson RA, Estey EH. Shortcomings in the clinical evaluation of new drugs: acute myeloid leukemia as paradigm. *Blood.* 2010
 101. Nguyen HQ, Hoffman-Liebermann B, Liebermann DA. The zinc finger transcription factor Egr-1 is essential for and restricts differentiation along the macrophage lineage. *Cell.* 1993; 72(2):197–209. [PubMed: 7678779]
 102. Shafarenko M, Liebermann DA, Hoffman B. Egr-1 abrogates the block imparted by c-Myc on terminal M1 myeloid differentiation. *Blood.* 2005; 106(3):871–878. [PubMed: 15840692]
 103. Soboloff J, Zhang Y, Minden M, Berger SA. Sensitivity of myeloid leukemia cells to calcium influx blockade: application to bone marrow purging. *Exp Hematol.* 2002; 30(10):1219–1226. [PubMed: 12384154]
 104. Inoue K, Tamaki H, Ogawa H, Oka Y, Soma T, Tatekawa T, Oji Y, Tsuboi A, Kim EH, Kawakami M, Akiyama T, Kishimoto T, Sugiyama H. Wilms' tumor gene (WT1) competes with differentiation-inducing signal in hematopoietic progenitor cells. *Blood.* 1998; 91(8):2969–2976. [PubMed: 9531608]
 105. Gardner JP, Balasubramanyam M, Studzinski GP. Up-regulation of Ca²⁺ influx mediated by store-operated channels in HL60 cells induced to differentiate by 1 alpha,25-dihydroxyvitamin D3. *J Cell Physiol.* 1997; 172(3):284–295. [PubMed: 9284948]
 106. Sekiya M, Adachi M, Hinoda Y, Imai K, Yachi A. Downregulation of Wilms' tumor gene (wt1) during myelomonocytic differentiation in HL60 cells. *Blood.* 1994; 83(7):1876–1882. [PubMed: 8142654]
 107. Wang Q, Salman H, Danilenko M, Studzinski GP. Cooperation between antioxidants and 1,25-dihydroxyvitamin D3 in induction of leukemia HL60 cell differentiation through the JNK/AP-1/Egr-1 pathway. *J Cell Physiol.* 2005; 204(3):964–974. [PubMed: 15799027]
 108. Vereb G Jr, Szollosi J, Matyus L, Balazs M, Hyun WC, Feuerstein BG. Depletion of intracellular calcium stores facilitates the influx of extracellular calcium in platelet derived growth factor stimulated A172 glioblastoma cells. *Cytometry.* 1996; 24(1):64–73. [PubMed: 8723904]
 109. Kovacs GG, Zsembery A, Anderson SJ, Komlosi P, Gillespie GY, Bell PD, Benos DJ, Fuller CM. Changes in intracellular Ca²⁺ and pH in response to thapsigargin in human glioblastoma cells and normal astrocytes. *Am J Physiol Cell Physiol.* 2005; 289(2):C361–C371. [PubMed: 15800052]
 110. Chigurupati S, Venkataraman R, Barrera D, Naganathan A, Madan M, Paul L, Pattisapu JV, Kyriazis GA, Sugaya K, Bushnev S, Lathia JD, Rich JN, Chan SL. Receptor channel TRPC6 is a key mediator of Notch-driven glioblastoma growth and invasiveness. *Cancer Res.* 2010; 70(1): 418–427. [PubMed: 20028870]
 111. Ding X, He Z, Zhou K, Cheng J, Yao H, Lu D, Cai R, Jin Y, Dong B, Xu Y, Wang Y. Essential Role of TRPC6 Channels in G2/M Phase Transition and Development of Human Glioma. *J Natl Cancer Inst.* 2010
 112. Wu H, Yang JM, Jin S, Zhang H, Hait WN. Elongation factor-2 kinase regulates autophagy in human glioblastoma cells. *Cancer Res.* 2006; 66(6):3015–3023. [PubMed: 16540650]

113. Szado T, Vanderheyden V, Parys JB, De Smedt H, Rietdorf K, Kotelevets L, Chastre E, Khan F, Landegren U, Soderberg O, Bootman MD, Roderick HL. Phosphorylation of inositol 1,4,5-trisphosphate receptors by protein kinase B/Akt inhibits Ca^{2+} release and apoptosis. *Proc Natl Acad Sci U S A*. 2008; 105(7):2427–2432. [PubMed: 18250332]
114. Yacoub A, Hamed HA, Allegood J, Mitchell C, Spiegel S, Lesniak MS, Ogretmen B, Dash R, Sarkar D, Broaddus WC, Grant S, Curiel DT, Fisher PB, Dent P. PERK-dependent regulation of ceramide synthase 6 and thioredoxin play a key role in mda-7/IL-24-induced killing of primary human glioblastoma multiforme cells. *Cancer Res*. 2010; 70(3):1120–1129. [PubMed: 20103619]
115. Scrideli CA, Carlotti CG Jr, Okamoto OK, Andrade VS, Cortez MA, Motta FJ, Lucio-Eterovic AK, Neder L, Rosemberg S, Oba-Shinjo SM, Tone LG. Gene expression profile analysis of primary glioblastomas and non-neoplastic brain tissue: identification of potential target genes by oligonucleotide microarray and real-time quantitative PCR. *J Neurooncol*. 2008; 88(3):281–291. [PubMed: 18398573]
116. Ruano Y, Mollejo M, Ribalta T, Fiano C, Camacho FI, Gomez E, de Lope AR, Hernandez-Moneo JL, Martinez P, Melendez B. Identification of novel candidate target genes in amplicons of Glioblastoma multiforme tumors detected by expression and CGH microarray profiling. *Mol Cancer*. 2006; 5:39. [PubMed: 17002787]
117. Liu C, Yao J, Mercola D, Adamson E. The transcription factor EGR-1 directly transactivates the fibronectin gene and enhances attachment of human glioblastoma cell line U251. *J Biol Chem*. 2000; 275(27):20315–20323. [PubMed: 10783396]
118. Huang RP, Liu C, Fan Y, Mercola D, Adamson ED. Egr-1 negatively regulates human tumor cell growth via the DNA-binding domain. *Cancer Res*. 1995; 55(21):5054–5062. [PubMed: 7585551]
119. Mittelbronn M, Harter P, Warth A, Lupescu A, Schilbach K, Vollmann H, Capper D, Goepfert B, Frei K, Bertalanffy H, Weller M, Meyermann R, Lang F, Simon P. EGR-1 is regulated by N-methyl-D-aspartate-receptor stimulation and associated with patient survival in human high grade astrocytomas. *Brain Pathol*. 2009; 19(2):195–204. [PubMed: 18489490]
120. Clark AJ, Ware JL, Chen MY, Graf MR, Van Meter TE, Dos Santos WG, Fillmore HL, Broaddus WC. Effect of WT1 gene silencing on the tumorigenicity of human glioblastoma multiforme cells. *J Neurosurg*. 2010; 112(1):18–25. [PubMed: 19392599]
121. Hashimoto N, Tsuboi A, Chiba Y, Izumoto S, Oka Y, Yoshimine T, Sugiyama H. Immunotherapy targeting the Wilms' tumor 1 gene product for patients with malignant brain tumors. *Brain Nerve*. 2009; 61(7):805–814. [PubMed: 19618858]
122. Nakahara Y, Okamoto H, Mineta T, Tabuchi K. Expression of the Wilms' tumor gene product WT1 in glioblastomas and medulloblastomas. *Brain Tumor Pathol*. 2004; 21(3):113–116. [PubMed: 15696971]
123. Schittenhelm J, Mittelbronn M, Nguyen TD, Meyermann R, Beschoner R. WT1 expression distinguishes astrocytic tumor cells from normal and reactive astrocytes. *Brain Pathol*. 2008; 18(3):344–353. [PubMed: 18371184]
124. Hashiba T, Izumoto S, Kagawa N, Suzuki T, Hashimoto N, Maruno M, Yoshimine T. Expression of WT1 protein and correlation with cellular proliferation in glial tumors. *Neurol Med Chir (Tokyo)*. 2007; 47(4):165–170. discussion 170. [PubMed: 17457020]
125. Flourakis M, Prevarskaya N. Insights into Ca^{2+} homeostasis of advanced prostate cancer cells. *Biochim Biophys Acta*. 2009; 1793(6):1105–1109. [PubMed: 19339216]
126. Gong Y, Blok LJ, Perry JE, Lindzey JK, Tindall DJ. Calcium regulation of androgen receptor expression in the human prostate cancer cell line LNCaP. *Endocrinology*. 1995; 136(5):2172–2178. [PubMed: 7720667]
127. Vanoverberghe K, Vanden Abeele F, Mariot P, Lepage G, Roudbaraki M, Bonnal JL, Mauroy B, Shuba Y, Skryma R, Prevarskaya N. Ca^{2+} homeostasis and apoptotic resistance of neuroendocrine-differentiated prostate cancer cells. *Cell Death Differ*. 2004; 11(3):321–330. [PubMed: 14685164]
128. Vanden Abeele F, Skryma R, Shuba Y, Van Coppenolle F, Slomianny C, Roudbaraki M, Mauroy B, Wuytack F, Prevarskaya N. Bcl-2-dependent modulation of Ca^{2+} homeostasis and store-operated channels in prostate cancer cells. *Cancer Cell*. 2002; 1(2):169–179. [PubMed: 12086875]

129. Yu J, de Belle I, Liang H, Adamson ED. Coactivating factors p300 and CBP are transcriptionally crossregulated by Egr1 in prostate cells, leading to divergent responses. *Mol Cell*. 2004; 15(1): 83–94. [PubMed: 15225550]
130. Abdulkadir SA, Carbone JM, Naughton CK, Humphrey PA, Catalona WJ, Milbrandt J. Frequent and early loss of the EGR1 corepressor NAB2 in human prostate carcinoma. *Hum Pathol*. 2001; 32(9):935–939. [PubMed: 11567222]
131. Svaren J, Ehrig T, Abdulkadir SA, Ehrenguber MU, Watson MA, Milbrandt J. EGR1 target genes in prostate carcinoma cells identified by microarray analysis. *J Biol Chem*. 2000; 275(49): 38524–38531. [PubMed: 10984481]
132. Furuya Y, Lundmo P, Short AD, Gill DL, Isaacs JT. The role of calcium, pH, and cell proliferation in the programmed (apoptotic) death of androgen-independent prostatic cancer cells induced by thapsigargin. *Cancer Res*. 1994; 54(23):6167–6175. [PubMed: 7954463]
133. Christensen SB, Andersen A, Kromann H, Treiman M, Tombal B, Denmeade S, Isaacs JT. Thapsigargin analogues for targeting programmed death of androgen-independent prostate cancer cells. *Bioorg Med Chem*. 1999; 7(7):1273–1280. [PubMed: 10465403]
134. Sohoel H, Jensen AM, Moller JV, Nissen P, Denmeade SR, Isaacs JT, Olsen CE, Christensen SB. Natural products as starting materials for development of second-generation SERCA inhibitors targeted towards prostate cancer cells. *Bioorg Med Chem*. 2006; 14(8):2810–2815. [PubMed: 16412648]
135. Picard C, McCarl CA, Papolos A, Khalil S, Luthy K, Hivroz C, LeDeist F, Rieux-Laucat F, Rechavi G, Rao A, Fischer A, Feske S. STIM1 mutation associated with a syndrome of immunodeficiency and autoimmunity. *N Engl J Med*. 2009; 360(19):1971–1980. [PubMed: 19420366]
136. Greenlee RT, Hill-Harmon MB, Murray T, Thun M. Cancer statistics, 2001. *CA Cancer J Clin*. 2001; 51(1):15–36. [PubMed: 11577478]
137. Auersperg N, Wong AS, Choi KC, Kang SK, Leung PC. Ovarian surface epithelium: biology, endocrinology, and pathology. *Endocr Rev*. 2001; 22(2):255–288. [PubMed: 11294827]
138. Salamanca CM, Maines-Bandiera SL, Leung PC, Hu YL, Auersperg N. Effects of epidermal growth factor/hydrocortisone on the growth and differentiation of human ovarian surface epithelium. *J Soc Gynecol Investig*. 2004; 11(4):241–251. S107155760400019X [pii].
139. Hohenstein P, Hastie ND. The many facets of the Wilms' tumour gene, WT1. *Hum Mol Genet*. 2006; 15(Spec No 2):R196–R201. [PubMed: 16987884]
140. Hosono S, Luo X, Hyink DP, Schnapp LM, Wilson PD, Burrow CR, Reddy JC, Atweh GF, Licht JD. WT1 expression induces features of renal epithelial differentiation in mesenchymal fibroblasts. *Oncogene*. 1999; 18(2):417–427. [PubMed: 9927198]
141. Saxen L, Sariola H. Early organogenesis of the kidney. *Pediatr Nephrol*. 1987; 1(3):385–392. [PubMed: 3153305]
142. Pritchard-Jones K, Fleming S, Davidson D, Bickmore W, Porteous D, Gosden C, Bard J, Buckler A, Pelletier J, Housman D, et al. The candidate Wilms' tumour gene is involved in genitourinary development. *Nature*. 1990; 346(6280):194–197. [PubMed: 2164159]
143. Kreidberg JA, Sariola H, Loring JM, Maeda M, Pelletier J, Housman D, Jaenisch R. WT-1 is required for early kidney development. *Cell*. 1993; 74(4):679–691. [PubMed: 8395349]
144. Shimizu M, Toki T, Takagi Y, Konishi I, Fujii S. Immunohistochemical detection of the Wilms' tumor gene (WT1) in epithelial ovarian tumors. *Int J Gynecol Pathol*. 2000; 19(2):158–163. [PubMed: 10782413]
145. Yamamoto S, Tsuda H, Kita T, Maekawa K, Fujii K, Kudoh K, Furuya K, Tamai S, Inazawa J, Matsubara O. Clinicopathological significance of WT1 expression in ovarian cancer: a possible accelerator of tumor progression in serous adenocarcinoma. *Virchows Arch*. 2007; 451(1):27–35. [PubMed: 17594113]
146. Barbolina MV, Adley BP, Shea LD, Stack MS. Wilms tumor gene protein 1 is associated with ovarian cancer metastasis and modulates cell invasion. *Cancer*. 2008; 112(7):1632–1641. [PubMed: 18260155]

147. Netinatsunthorn W, Hanprasertpong J, Dechsukhum C, Leetanaporn R, Geater A. WT1 gene expression as a prognostic marker in advanced serous epithelial ovarian carcinoma: an immunohistochemical study. *BMC Cancer*. 2006; 6:90. [PubMed: 16606472]
148. Hylander B, Repasky E, Shrikant P, Intengan M, Beck A, Driscoll D, Singhal P, Lele S, Odunsi K. Expression of Wilms tumor gene (WT1) in epithelial ovarian cancer. *Gynecol Oncol*. 2006; 101(1):12–17. [PubMed: 16263157]
149. Huber SM, Braun GS, Segerer S, Veh RW, Horster MF. Metanephrogenic mesenchyme-to-epithelium transition induces profound expression changes of ion channels. *Am J Physiol Renal Physiol*. 2000; 279(1):F65–F76. [PubMed: 10894788]
150. Cullen PJ, Lockyer PJ. Integration of calcium and Ras signalling. *Nat Rev Mol Cell Biol*. 2002; 3(5):339–348. nrm808 [pii]. [PubMed: 11988768]
151. Schmidt CR, Washington MK, Gi YJ, Coffey RJ, Beauchamp RD, Pearson AS. Dysregulation of E-cadherin by oncogenic Ras in intestinal epithelial cells is blocked by inhibiting MAP kinase. *Am J Surg*. 2003; 186(5):426–430. [PubMed: 14599601]
152. Christofori G, Semb H. The role of the cell-adhesion molecule E-cadherin as a tumour-suppressor gene. *Trends Biochem Sci*. 1999; 24(2):73–76. [PubMed: 10098402]
153. Sawada K, Mitra AK, Radjabi AR, Bhaskar V, Kistner EO, Tretiakova M, Jagadeeswaran S, Montag A, Becker A, Kenny HA, Peter ME, Ramakrishnan V, Yamada SD, Lengyel E. Loss of E-cadherin promotes ovarian cancer metastasis via alpha 5-integrin, which is a therapeutic target. *Cancer Res*. 2008; 68(7):2329–2339. 10.1158/0008-5472.CAN-07-5167. [PubMed: 18381440]

Fig. 1. Model depicting control of Ca^{2+} signaling and homeostasis in non-excitable cells
 Activation of Phospholipase C (PLC)-coupled receptors initiates a signaling cascade wherein newly generated inositol 1,4,5-triphosphate (IP_3) binds to and activates its receptor (IP_3R) located on the endoplasmic reticulum (ER) membrane. Once activated, the IP_3R releases ER Ca^{2+} content into the cytoplasm. This resultant ER Ca^{2+} depletion is sensed by STIM proteins, which aggregate near the plasma membrane (PM) where it interacts with Orai1, causing store-operated Ca^{2+} entry. Ca^{2+} /ATPases located on both the ER (SERCA, sarco/endoplasmic calcium ATPase) and PM (PMCA, plasma membrane calcium ATPase) rapidly remove cytosolic Ca^{2+} , resulting in recovery of both ER and cytosolic Ca^{2+} concentration.

Fig. 2. Regulation of STIM1 expression by WT1 and EGR1

Early Growth Response 1 (EGR1) activation is achieved through multiple receptor-dependent signal transduction pathways downstream of both the Ras oncogene (via the MAPK pathway) and Phospholipase C (PLC; via protein kinase C). Activated EGR1 rapidly enters the nucleus and binds to 2 response elements on the STIM1 gene sequences to induce its expression. However, Wilms Tumor Suppressor 1 (WT1) inhibits binding of EGR1 at the first response element, thereby repressing STIM1 expression. However, changes in STIM1 expression affect cytosolic Ca^{2+} concentration, thereby indirectly modulating EGR1 expression via protein kinase C (PKC)-, protein kinase A (PKA)- and calmodulin kinase (CamK)-dependent pathways.