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WT1/EGR1-mediated control of STIM1 expression and function in cancer cells

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TABLE OF CONTENTS

1. Abstract
2. Introduction
- *2.1. Bcl2-mediated control of ER Ca^{2+} content in cancer cells*
- *2.2. TRP cation channel function in cancer cells*
- *2.3. Molecular Mechanisms of Ca^{2+} entry*
- *2.4. The zinc finger transcription factors WT1 and EGR1*

3. WT1 and EGR1: Oncogenes/tumor suppressors regulating Ca^{2+} homeostasis
- *3.1. WT1 as a negative regulator of STIM1 expression in Wilms Tumor*
- *3.2. WT1, EGR1 and STIM1 in breast cancer*
- *3.3. WT1, EGR1 and Ca^{2+} signaling in Acute Myeloid Leukemia*
- *3.4. EGR1 and STIM1 expression in Glioblastoma*
- *3.5. EGR1-mediated control of SOCe in prostate cancer*
- *3.6. WT1/EGR1 and ovarian carcinoma*

4. Summary and perspective
5. Acknowledgements
6. References

1. ABSTRACT

There have been numerous publications linking Ca^{2+} signaling and cancer, however, a clear explanation for this link 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 (Figure 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

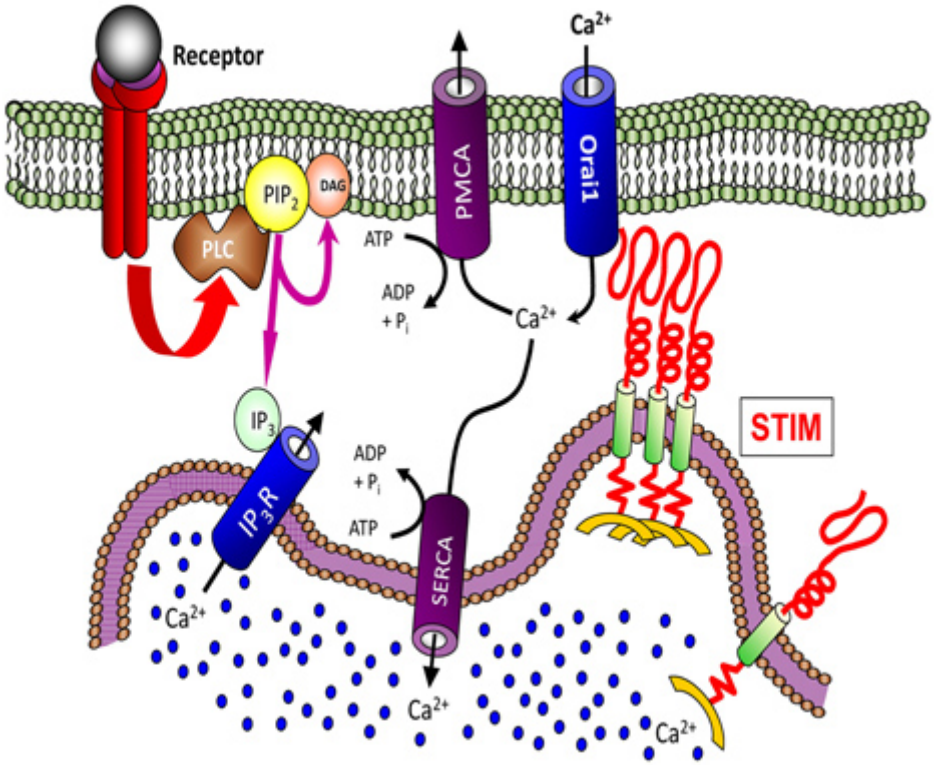

Figure 1. Model depicting control of Ca^{2+} signaling and homeostasis in non-excitable cells. Activation of Phospholipase C (PLC)-coupled receptors initiates a signaling cascade wherein newly generated inositol 1,4,5-triphosphate (IP_3) binds to and activates its receptor (IP_3R) located on the endoplasmic 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.

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 level 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 of 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 one of 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 therapeutic 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 Ca^{2+} 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 Ca^{2+} 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 5 AML cell lines examined. Although 3 out 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 vitamin D3-induced differentiation into monocytes leads to SOCe recovery (105), loss of WT1 expression (106) and EGR1 induction (107). Precisely how these differential Ca^{2+} 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 SOCe 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 that glioblastoma cell survival is enhanced by decreasing ER Ca^{2+} release via Akt-mediated inhibition of $InsP_3R$ 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 revealed 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 PDGFα 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 SOCe 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-knockout 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 Orai1 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

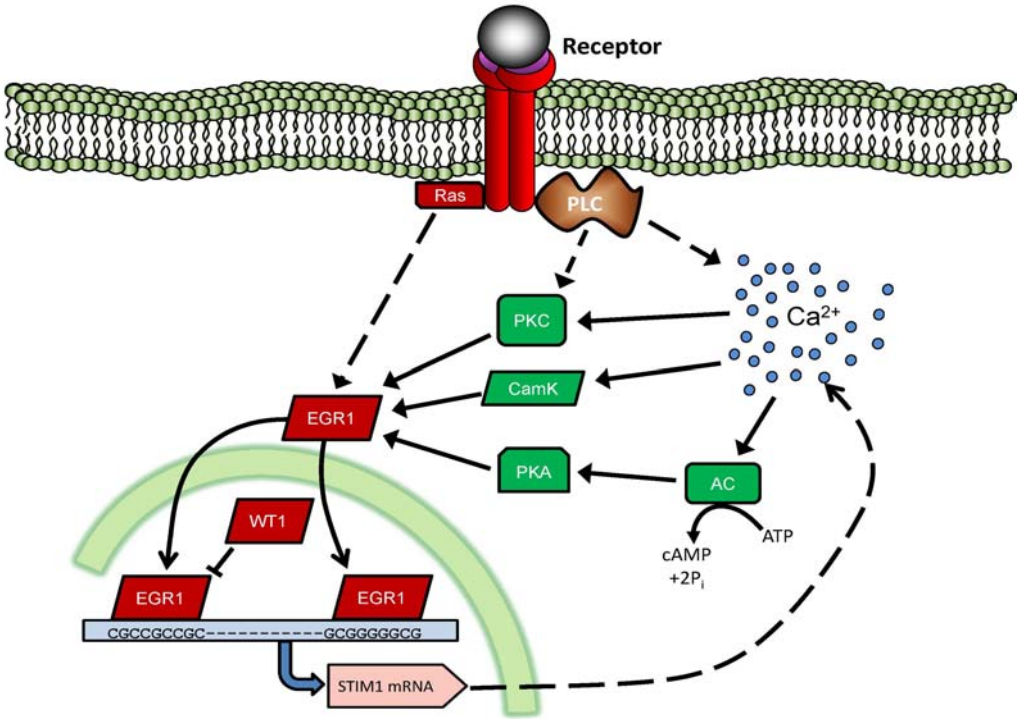

Figure 2. Regulation of STIM1 expression by WT1 and EGR1. Early Growth Response 1 (EGR1) activation is achieved through multiple receptor-dependent signal transduction pathways downstream of both the Ras oncogene (via the MAPK pathway) and Phospholipase C (PLC; via protein kinase C). Activated EGR1 rapidly 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.

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 shear variety of the signaling pathways in control of EGR1 expression (Figure 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.

5. ACKNOWLEDGEMENTS

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6. REFERENCES

1.A. L. Boynton, J. F. Whitfield, R. J. Isaacs and H. J. Morton: Control of 3T3 cell proliferation by calcium. *In vitro*, 10, 12-7 (1974)

2.J. F. Whitfield, A. L. Boynton, J. P. MacManus, M. Sikorska and B. K. Tsang: The regulation of cell proliferation by calcium and cyclic AMP. *Mol Cell Biochem*, 27(3), 155-79 (1979)

3.M. J. Berridge and R. F. Irvine: Inositol trisphosphate, a novel second messenger in cellular signal transduction. *Nature*, 312(5992), 315-21 (1984)

4.K. Bridges, R. Levenson, D. Housman and L. Cantley: Calcium regulates the commitment of murine erythroleukemia cells to terminal erythroid differentiation. *J Cell Biol*, 90(2), 542-4 (1981)

5.J. Holliday, R. J. Adams, T. J. Sejnowski and N. C. Spitzer: Calcium-induced release of calcium regulates differentiation of cultured spinal neurons. *Neuron*, 7(5), 787-96 (1991)

6.S. Orrenius, B. Zhivotovsky and P. Nicotera: Regulation of cell death: the calcium-apoptosis link. *Nat Rev Mol Cell Biol*, 4(7), 552-65 (2003)

7.A. L. Boynton and J. F. Whitfield: Different calcium requirements for proliferation of conditionally and unconditionally tumorigenic mouse cells. *Proc Natl Acad Sci U S A*, 73(5), 1651-4 (1976)

8.A. L. Boynton and J. F. Whitfield: Calcium requirements for the proliferation of cells infected with a temperature-

sensitive mutant of Rous sarcoma virus. *Cancer Res*, 38(5), 1237-40 (1978)

9.J. F. Whitfield: Calcium signals and cancer. *Crit Rev Oncog*, 3(1-2), 55-90 (1992)

10.J. M. Adams and S. Cory: The Bcl-2 apoptotic switch in cancer development and therapy. *Oncogene*, 26(9), 1324-37 (2007)

11.A. Frenzel, F. Grespi, W. Chmelewskij and A. Villunger: Bcl2 family proteins in carcinogenesis and the treatment of cancer. *Apoptosis*, 14(4), 584-96 (2009)

12.E. Lomonosova and G. Chinnadurai: BH3-only proteins in apoptosis and beyond: an overview. *Oncogene*, 27 Suppl 1, S2-19 (2008)

13.E. F. Eckenrode, J. Yang, G. V. Velmurugan, J. K. Foskett and C. White: Apoptosis protection by Mcl-1 and Bcl-2 modulation of inositol 1,4,5-trisphosphate receptor-dependent Ca^{2+} signaling. *J Biol Chem*, 285(18), 13678-84 (2010)

14.C. Li, X. Wang, H. Vais, C. B. Thompson, J. K. Foskett and C. White: 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*, 104(30), 12565-70 (2007)

15.C. White, C. Li, J. Yang, N. B. Petrenko, M. Madesh, C. B. Thompson and J. K. Foskett: The endoplasmic reticulum gateway to apoptosis by Bcl-X(L) modulation of the $InsP_3R$. *Nat Cell Biol*, 7(10), 1021-8 (2005)

16.P. Pinton, D. Ferrari, P. Magalhaes, K. Schulze-Osthoff, F. Di Virgilio, T. Pozzan and R. Rizzuto: Reduced loading of intracellular Ca(2+) stores and downregulation of capacitative Ca(2+) influx in Bcl-2-overexpressing cells. *J Cell Biol*, 148(5), 857-62 (2000)

17.R. Foyouzi-Youssefi, S. Arnaudeau, C. Borner, W. L. Kelley, J. Tschopp, D. P. Lew, N. Demaurex and K. H. Krause: Bcl-2 decreases the free Ca^{2+} concentration within the endoplasmic reticulum. *Proc Natl Acad Sci U S A*, 97(11), 5723-8 (2000)

18.Y. P. Rong, G. Bultynck, A. S. Aromolaran, F. Zhong, J. B. Parys, H. De Smedt, G. A. Mignery, H. L. Roderick, M. D. Bootman and C. W. Distelhorst: The BH4 domain of Bcl-2 inhibits ER calcium release and apoptosis by binding the regulatory and coupling domain of the IP3 receptor. *Proc Natl Acad Sci U S A*, 106(34), 14397-402 (2009)

19.Y. P. Rong, A. S. Aromolaran, G. Bultynck, F. Zhong, X. Li, K. McColl, S. Matsuyama, S. Herlitze, H. L. Roderick, M. D. Bootman, G. A. Mignery, J. B. Parys, H. De Smedt and C. W. Distelhorst: Targeting Bcl-2-IP3 receptor interaction to reverse Bcl-2's inhibition of apoptotic calcium signals. *Mol Cell*, 31(2), 255-65 (2008)

20.S. A. Oakes, L. Scorrano, J. T. Opferman, M. C. Bassik, M. Nishino, T. Pozzan and S. J. Korsmeyer: 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*, 102(1), 105-10 (2005)

21.K. Venkatachalam and C. Montell: TRP channels. *Annu Rev Biochem*, 76, 387-417 (2007)

22.L. M. Duncan, J. Deeds, J. Hunter, J. Shao, L. M. Holmgren, E. A. Woolf, R. I. Tepper and A. W. Shyjan: Down-regulation of the novel gene melastatin correlates with potential for melanoma metastasis. *Cancer Res*, 58(7), 1515-20 (1998)

23.L. Tsavaler, M. H. Shapero, S. Morkowski and R. Laus: 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*, 61(9), 3760-9 (2001)

24.J. B. Peng, L. Zhuang, U. V. Berger, R. M. Adam, B. J. Williams, E. M. Brown, M. A. Hediger and M. R. Freeman: CaT1 expression correlates with tumor grade in prostate cancer. *Biochem Biophys Res Commun*, 282(3), 729-34 (2001)

25.V. Lehen'kyi, M. Flourakis, R. Skryma and N. Prevarskaya: TRPV6 channel controls prostate cancer cell proliferation via Ca(2+)/NFAT-dependent pathways. *Oncogene*, 26(52), 7380-5 (2007)

26.C. El Boustany, G. Bidaux, A. Enfissi, P. Delcourt, N. Prevarskaya and T. Capiod: Capacitative calcium entry and transient receptor potential canonical 6 expression control human hepatoma cell proliferation. *Hepatology*, 47(6), 2068-77 (2008)

27.J. W. Putney, Jr.: A model for receptor-regulated calcium entry. *Cell Calcium*, 7(1), 1-12 (1986)

28.J. Liou, M. L. Kim, W. D. Heo, J. T. Jones, J. W. Myers, J. E. Ferrell, Jr. and T. Meyer: STIM is a Ca^{2+} sensor essential for Ca^{2+}-store-depletion-triggered Ca^{2+} influx. *Curr Biol*, 15(13), 1235-41 (2005)

29.J. Roos, P. J. Digregorio, A. V. Yeromin, K. Ohlsen, M. Lioudyno, S. Zhang, O. Safrina, J. A. Kozak, S. L. Wagner, M. D. Cahalan, G. Velicelebi and K. A. Stauderman: STIM1, an essential and conserved component of store-operated Ca^{2+} channel function. *J Cell Biol*, 169(3), 435-45 (2005)

30.S. Feske, Y. Gwack, M. Prakriya, S. Srikanth, S. H. Puppel, B. Tanasa, P. G. Hogan, R. S. Lewis, M. Daly and A. Rao: A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. *Nature*, 441(7090), 179-85 (2006)

31.M. Vig, C. Peinelt, A. Beck, D. L. Koomoa, D. Rabah, M. Koblan-Huberson, S. Kraft, H. Turner, A. Fleig, R. Penner and J. P. Kinet: CRACM1 is a plasma membrane protein essential for store-operated Ca^{2+} entry. *Science*, 312(5777), 1220-3 (2006)

32.S. L. Zhang, A. V. Yeromin, X. H. Zhang, Y. Yu, O. Safrina, A. Penna, J. Roos, K. A. Stauderman and M. D. Cahalan: 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(24), 9357-62 (2006)

33.T. Hewavitharana, X. Deng, J. Soboloff and D. L. Gill: Role of STIM and Orai proteins in the store-operated calcium signaling pathway. *Cell Calcium*, 42(2), 173-82 (2007)

34.R. M. Luik and R. S. Lewis: New insights into the molecular mechanisms of store-operated Ca^{2+} signaling in T cells. *Trends Mol Med*, 13(3), 103-7 (2007)

35.J. Soboloff, M. A. Spassova, X. D. Tang, T. Hewavitharana, W. Xu and D. L. Gill: Orai1 and STIM Reconstitute Store-operated Calcium Channel Function. *J Biol Chem*, 281(30), 20661-5 (2006)

36.S. Feske: Calcium signalling in lymphocyte activation and disease. *Nat Rev Immunol*, 7(9), 690-702 (2007)

37.P. G. Hogan and A. Rao: Dissecting ICRAC, a store-operated calcium current. *Trends Biochem Sci*, 32(5), 235-45 (2007)

38.X. Deng, Y. Wang, Y. Zhou, J. Soboloff and D. L. Gill: STIM and Orai: dynamic intermembrane coupling to control cellular calcium signals. *J Biol Chem*, 284(34), 22501-5 (2009)

39.R. T. Williams, S. S. Manji, N. J. Parker, M. S. Hancock, L. Van Stekelenburg, J. P. Eid, P. V. Senior, J. S. Kazenwadel, T. Shandala, R. Saint, P. J. Smith and M. A. Dziadek: Identification and characterization of the STIM (stromal interaction molecule) gene family: coding for a novel class of transmembrane proteins. *Biochem J*, 357(Pt 3), 673-85 (2001)

40.P. B. Stathopulos, L. Zheng, G. Y. Li, M. J. Plevin and M. Ikura: Structural and mechanistic insights into STIM1-mediated initiation of store-operated calcium entry. *Cell*, 135(1), 110-22 (2008)

41.P. B. Stathopulos, G. Y. Li, M. J. Plevin, J. B. Ames and M. Ikura: Stored Ca^{2+} depletion-induced oligomerization of stromal interaction molecule 1 (STIM1) via the EF-SAM region: An initiation mechanism for capacitive Ca^{2+} entry. *J Biol Chem*, 281(47), 35855-62 (2006)

42.P. B. Stathopulos, L. Zheng and M. Ikura: Stromal Interaction Molecule (STIM) 1 and STIM2 Calcium Sensing Regions Exhibit Distinct Unfolding and Oligomerization Kinetics. *J Biol Chem*, 284(2), 728-32 (2009)

43.T. Hewavitharana, X. Deng, Y. Wang, M. F. Ritchie, G. V. Girish, J. Soboloff and D. L. Gill: Location and function of STIM1 in the activation of Ca^{2+} entry signals. *J Biol Chem*, 283(38), 26252-62 (2008)

44.R. M. Luik, B. Wang, M. Prakriya, M. M. Wu and R. S. Lewis: Oligomerization of STIM1 couples ER calcium depletion to CRAC channel activation. *Nature*, 454(7203), 538-42 (2008)

45.M. M. Wu, J. Buchanan, R. M. Luik and R. S. Lewis: Ca^{2+} store depletion causes STIM1 to accumulate in ER regions closely associated with the plasma membrane. *J Cell Biol*, 174(6), 803-13 (2006)

46.R. M. Luik, M. M. Wu, J. Buchanan and R. S. Lewis: The elementary unit of store-operated Ca^{2+} entry: local activation of CRAC channels by STIM1 at ER-plasma membrane junctions. *J Cell Biol*, 174(6), 815-25 (2006)

47.Y. Wang, X. Deng, Y. Zhou, E. Hendron, S. Mancarella, M. F. Ritchie, X. D. Tang, Y. Baba, T. Kurosaki, Y. Mori, J. Soboloff and D. L. Gill: STIM protein coupling in the activation of Orai channels. *Proc Natl Acad Sci U S A*, 106(18), 7391-6 (2009)

48.M. Muik, M. Fahrner, I. Derler, R. Schindl, J. Bergsmann, I. Frischauf, K. Groschner and C. Romanin: A Cytosolic Homomerization and a Modulatory Domain within STIM1 C Terminus Determine Coupling to ORAI1 Channels. *J Biol Chem*, 284(13), 8421-6 (2009)

49.C. Y. Park, P. J. Hoover, F. M. Mullins, P. Bachhawat, E. D. Covington, S. Raunser, T. Walz, K. C. Garcia, R. E. Dolmetsch and R. S. Lewis: STIM1 clusters and activates CRAC channels via direct binding of a cytosolic domain to Orai1. *Cell*, 136(5), 876-90 (2009)

50.J. P. Yuan, W. Zeng, M. R. Dorwart, Y. J. Choi, P. F. Worley and S. Muallem: SOAR and the polybasic STIM1 domains gate and regulate Orai channels. *Nat Cell Biol*, 11(3), 337-43 (2009)

51.J. C. Mercer, W. I. Dehaven, J. T. Smyth, B. Wedel, R. R. Boyles, G. S. Bird and J. W. Putney, Jr.: Large store-operated calcium selective currents due to co-expression of Orai1 or Orai2 with the intracellular calcium sensor, Stim1. *J Biol Chem*, 281(34), 24979-90 (2006)

52.C. Peinelt, M. Vig, D. L. Koomoa, A. Beck, M. J. Nadler, M. Koblan-Huberson, A. Lis, A. Fleig, R. Penner and J. P. Kinet: Amplification of CRAC current by STIM1 and CRACM1 (Orai1). *Nat Cell Biol*, 8(7), 771-3 (2006)

53.O. Brandman, J. Liou, W. S. Park and T. Meyer: STIM2 is a feedback regulator that stabilizes basal cytosolic and endoplasmic reticulum Ca^{2+} levels. *Cell*, 131(7), 1327-39 (2007)

54.S. Parvez, A. Beck, C. Peinelt, J. Soboloff, A. Lis, M. Monteilh-Zoller, D. L. Gill, A. Fleig and R. Penner: STIM2 protein mediates distinct store-dependent and store-independent modes of CRAC channel activation. *Faseb J*, 22(3), 752-61 (2008)

55.J. Soboloff, M. A. Spassova, T. Hewavitharana, L. P. He, W. Xu, L. S. Johnstone, M. A. Dziadek and D. L. Gill:

STIM2 Is an Inhibitor of STIM1-Mediated Store-Operated Ca(2+) Entry. *Curr Biol*, 16(14), 1465-70 (2006)

56.Y. Zhou, S. Mancarella, Y. Wang, C. Yue, M. Ritchie, D. L. Gill and J. Soboloff: The Short N-terminal Domains of STIM1 and STIM2 Control the Activation Kinetics of Orai1 Channels. *J Biol Chem*, 284(29), 19164-8 (2009)

57.W. I. DeHaven, J. T. Smyth, R. R. Boyles and J. W. Putney, Jr.: Calcium inhibition and calcium potentiation of Orai1, Orai2, and Orai3 calcium release-activated calcium channels. *J Biol Chem*, 282(24), 17548-56 (2007)

58.A. Lis, C. Peinelt, A. Beck, S. Parvez, M. Monteilh-Zoller, A. Fleig and R. Penner: CRACM1, CRACM2, and CRACM3 are store-operated Ca2+ channels with distinct functional properties. *Curr Biol*, 17(9), 794-800 (2007)

59.M. F. Ritchie, C. Yue, Y. Zhou, P. J. Houghton and J. Soboloff: Wilms Tumor Suppressor 1 (WT1) and Early Growth Response 1 (EGR1) Are Regulators of STIM1 Expression. *J Biol Chem*, 285(14), 10591-6 (2010)

60.A. M. Beckmann, I. Matsumoto and P. A. Wilce: AP-1 and Egr DNA-binding activities are increased in rat brain during ethanol withdrawal. *J Neurochem*, 69(1), 306-14 (1997)

61.A. Gashler and V. P. Sukhatme: Early growth response protein 1 (Egr-1): prototype of a zinc-finger family of transcription factors. *Prog Nucleic Acid Res Mol Biol*, 50, 191-224 (1995)

62.C. Wang, S. Dostanic, N. Servant and L. E. Chalifour: Egr-1 negatively regulates expression of the sodium-calcium exchanger-1 in cardiomyocytes *in vitro* and *in vivo*. *Cardiovasc Res*, 65(1), 187-94 (2005)

63.A. Kasneci, N. M. Kemeny-Suss, S. V. Komarova and L. E. Chalifour: Egr-1 negatively regulates calsequestrin expression and calcium dynamics in ventricular cells. *Cardiovasc Res*, 81(4), 695-702 (2009)

64.M. Brady, M. U. Koban, K. A. Dellow, M. Yacoub, K. R. Boheler and S. J. Fuller: Sp1 and Sp3 transcription factors are required for trans-activation of the human SERCA2 promoter in cardiomyocytes. *Cardiovasc Res*, 60(2), 347-54 (2003)

65.S. Hara, M. Arai, K. Tomaru, H. Doi, N. Koitabashi, T. Iso, A. Watanabe, T. Tanaka, T. Maeno, T. Suga, T. Yokoyama and M. Kurabayashi: Prostaglandin F2alpha inhibits SERCA2 gene transcription through an induction of Egr-1 in cultured neonatal rat cardiac myocytes. *Int Heart J*, 49(3), 329-42 (2008)

66.M. Arai, A. Yoguchi, T. Takizawa, T. Yokoyama, T. Kanda, M. Kurabayashi and R. Nagai: Mechanism of doxorubicin-induced inhibition of sarcoplasmic reticulum Ca(2+)-ATPase gene transcription. *Circ Res*, 86(1), 8-14 (2000)

67.Y. Zhou, M. F. Ritchie, C. Yue, M. Madesh and J. Soboloff: Targeting Store-operated Ca^{2+} Entry to Induce Cell Death depends on WT1-Regulated SERCA2 expression. *Cell Death Differ*, in revision (2010)

68.V. Scharnhorst, A. L. Menke, J. Attema, J. K. Haneveld, N. Riteco, G. J. van Steenbrugge, A. J. van der Eb and A. G. Jochemsen: EGR-1 enhances tumor growth and modulates the effect of the Wilms' tumor 1 gene products on tumorigenicity. *Oncogene*, 19(6), 791-800 (2000)

69.S. K. Bae, M. H. Bae, M. Y. Ahn, M. J. Son, Y. M. Lee, M. K. Bae, O. H. Lee, B. C. Park and K. W. Kim: Egr-1 mediates transcriptional activation of IGF-II gene in response to hypoxia. *Cancer Res*, 59(23), 5989-94 (1999)

70.T. B. Hamilton, F. Borel and P. J. Romaniuk: Comparison of the DNA binding characteristics of the related zinc finger proteins WT1 and EGR1. *Biochemistry*, 37(7), 2051-8 (1998)

71.A. A. Morrison, R. L. Viney and M. R. Ladomery: The post-transcriptional roles of WT1, a multifunctional zinc-finger protein. *Biochim Biophys Acta*, 1785(1), 55-62 (2008)

72.E. Nurmemmedov, R. K. Yengo, H. Uysal, R. Karlsson and M. M. Thunnissen: New insights into DNA-binding behavior of Wilms tumor protein (WT1)--a dual study. *Biophys Chem*, 145(2-3), 116-25 (2009)

73.D. J. Richard, V. Schumacher, B. Royer-Pokora and S. G. Roberts: Par4 is a coactivator for a splice isoform-specific transcriptional activation domain in WT1. *Genes Dev*, 15(3), 328-39 (2001)

74.J. D. Gibbs, D. A. Liebermann and B. Hoffman: Leukemia suppressor function of Egr-1 is dependent on transforming oncogene. *Leukemia*, 22(10), 1909-16 (2008)

75.K. L. Redmond, N. T. Crawford, H. Farmer, Z. C. D'Costa, G. J. O'Brien, N. E. Buckley, R. D. Kennedy, P. G. Johnston, D. P. Harkin and P. B. Mullan: T-box 2 represses NDRG1 through an EGR1-dependent mechanism to drive the proliferation of breast cancer cells. *Oncogene*, 29(22), 3252-62 (2010)

76.S. A. Abdulkadir, Z. Qu, E. Garabedian, S. K. Song, T. J. Peters, J. Svaren, J. M. Carbone, C. K. Naughton, W. J. Catalona, J. J. Ackerman, J. I. Gordon, P. A. Humphrey and J. Milbrandt: Impaired prostate tumorigenesis in Egr1-deficient mice. *Nat Med*, 7(1), 101-7 (2001)

77.M. N. Rivera and D. A. Haber: Wilms' tumour: connecting tumorigenesis and organ development in the kidney. *Nat Rev Cancer*, 5(9), 699-712 (2005)

78.C. Owen, J. Fitzgibbon and P. Paschka: The clinical relevance of Wilms Tumour 1 (WT1) gene mutations in acute leukaemia. *Hematol Oncol*, 28(1), 13-9 (2010)

79.L. Yang, Y. Han, F. Suarez Saiz and M. D. Minden: A tumor suppressor and oncogene: the WT1 story. *Leukemia*, 21(5), 868-76 (2007)

80.J. L. Gregg, K. E. Brown, E. M. Mintz, H. Piontkivska and G. C. Fraizer: Analysis of gene expression in prostate cancer epithelial and interstitial stromal cells using laser capture microdissection. *BMC Cancer*, 10, 165 (2010)

81.V. Baron, E. D. Adamson, A. Calogero, G. Ragona and D. Mercola: The transcription factor Egr1 is a direct regulator of multiple tumor suppressors including TGFbeta1, PTEN, p53, and fibronectin. *Cancer Gene Ther*, 13(2), 115-24 (2006)

82.P. Zapata-Benavides, M. Tuna, G. Lopez-Berestein and A. M. Tari: Downregulation of Wilms' tumor 1 protein inhibits breast cancer proliferation. *Biochem Biophys Res Commun*, 295(4), 784-90 (2002)

83.G. B. Silberstein, K. Van Horn, P. Strickland, C. T. Roberts, Jr. and C. W. Daniel: Altered expression of the WT1 wilms tumor suppressor gene in human breast cancer. *Proc Natl Acad Sci U S A*, 94(15), 8132-7 (1997)

84.Y. Miyoshi, A. Ando, C. Egawa, T. Taguchi, Y. Tamaki, H. Tamaki, H. Sugiyama and S. Noguchi: High expression of Wilms' tumor suppressor gene predicts poor prognosis in breast cancer patients. *Clin Cancer Res*, 8(5), 1167-71 (2002)

85.K. Kaufmann and G. Thiel: 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*, 189(1-2), 83-91 (2001)

86.M. Takahashi, X. J. Yang, T. T. Lavery, K. A. Furge, B. O. Williams, M. Tretiakova, A. Montag, N. J. Vogelzang, G. G. Re, A. J. Garvin, S. Soderhall, S. Kagawa, D. Hazel-Martin, A. Nordenskjold and B. T. Teh: Gene expression profiling of favorable histology Wilms tumors and its correlation with clinical features. *Cancer Res*, 62(22), 6598-605 (2002)

87.A. J. Garvin, G. G. Re, B. I. Tarnowski, D. J. Hazen-Martin and D. A. Sens: 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*, 142(2), 375-80 (1993)

88.S. Sabbioni, G. Barbanti-Brodano, C. M. Croce and M. Negrini: GOK: a gene at 11p15 involved in rhabdomyosarcoma and rhabdoid tumor development. *Cancer Res*, 57(20), 4493-7 (1997)

89.S. Sabbioni, A. Veronese, M. Trubia, R. Taramelli, G. Barbanti-Brodano, C. M. Croce and M. Negrini: 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*, 86(3-4), 214-8 (1999)

90.P. J. Houghton, C. L. Morton, C. Tucker, D. Payne, E. Favours, C. Cole, R. Gorlick, E. A. Kolb, W. Zhang, R. Lock, H. Carol, M. Tajbakhsh, C. P. Reynolds, J. M. Maris, J. Courtright, S. T. Keir, H. S. Friedman, C. Stopford, J. Zeidner, J. Wu, T. Liu, C. A. Billups, J. Khan, S. Ansher, J. Zhang and M. A. Smith: The pediatric preclinical testing program: description of models and early testing results. *Pediatr Blood Cancer*, 49(7), 928-40 (2007)

91.R. Natrajan, S. E. Little, J. S. Reis-Filho, L. Hing, B. Messahel, P. E. Grundy, J. S. Dome, T. Schneider, G. M. Vujanic, K. Pritchard-Jones and C. Jones: Amplification and overexpression of CACNA1E correlates with relapse in favorable histology Wilms' tumors. *Clin Cancer Res*, 12(24), 7284-93 (2006)

92.K. Pritchard-Jones and J. Pritchard: Success of clinical trials in childhood Wilms' tumour around the world. *Lancet*, 364(9444), 1468-70 (2004)

93.S. Wittmann, C. Wunder, B. Zirn, R. Furtwangler, J. Wegert, N. Graf and M. Gessler: New prognostic markers revealed by evaluation of genes correlated with clinical parameters in Wilms tumors. *Genes Chromosomes Cancer*, 47(5), 386-95 (2008)

94.T. F. Zhang, S. Q. Yu, L. S. Guan and Z. Y. Wang: Inhibition of breast cancer cell growth by the Wilms' tumor suppressor WT1 is associated with a destabilization of beta-catenin. *Anticancer Res*, 23(5A), 3575-84 (2003)

95.N. Reizner, S. Maor, R. Sarfstein, S. Abramovitch, W. V. Welshons, E. M. Curran, A. V. Lee and H. Werner: 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*, 35(1), 135-44 (2005)

96.R. K. Motiani, I. F. Abdullaev and M. Trebak: 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*, 285(25), 19173-83 (2010)

97.D. M. Loeb, E. Evron, C. B. Patel, P. M. Sharma, B. Niranjan, L. Buluwela, S. A. Weitzman, D. Korz and S. Sukumar: Wilms' tumor suppressor gene (WT1) is expressed in primary breast tumors despite tumor-specific promoter methylation. *Cancer Res*, 61(3), 921-5 (2001)

98.K. Ronski, M. Sanders, J. A. Burleson, V. Moyo, P. Benn and M. Fang: Early growth response gene 1 (EGR1) is deleted in estrogen receptor-negative human breast carcinoma. *Cancer*, 104(5), 925-30 (2005)

99.S. Yang, J. J. Zhang and X. Y. Huang: Orai1 and STIM1 are critical for breast tumor cell migration and metastasis. *Cancer Cell*, 15(2), 124-34 (2009)

100.R. B. Walter, F. R. Appelbaum, M. S. Tallman, N. S. Weiss, R. A. Larson and E. H. Estey: Shortcomings in the

clinical evaluation of new drugs: acute myeloid leukemia as paradigm. *Blood* (2010)

101.H. Q. Nguyen, B. Hoffman-Liebermann and D. A. Liebermann: The zinc finger transcription factor Egr-1 is essential for and restricts differentiation along the macrophage lineage. *Cell*, 72(2), 197-209 (1993)

102.M. Shafarenko, D. A. Liebermann and B. Hoffman: Egr-1 abrogates the block imparted by c-Myc on terminal M1 myeloid differentiation. *Blood*, 106(3), 871-8 (2005)

103.J. Soboloff, Y. Zhang, M. Minden and S. A. Berger: Sensitivity of myeloid leukemia cells to calcium influx blockade: application to bone marrow purging. *Exp Hematol*, 30(10), 1219-26 (2002)

104.K. Inoue, H. Tamaki, H. Ogawa, Y. Oka, T. Soma, T. Tatekawa, Y. Oji, A. Tsuboi, E. H. Kim, M. Kawakami, T. Akiyama, T. Kishimoto and H. Sugiyama: Wilms' tumor gene (WT1) competes with differentiation-inducing signal in hematopoietic progenitor cells. *Blood*, 91(8), 2969-76 (1998)

105.J. P. Gardner, M. Balasubramanyam and G. P. Studzinski: Up-regulation of Ca^{2+} influx mediated by store-operated channels in HL60 cells induced to differentiate by 1 alpha,25-dihydroxyvitamin D3. *J Cell Physiol*, 172(3), 284-95 (1997)

106.M. Sekiya, M. Adachi, Y. Hinoda, K. Imai and A. Yachi: Downregulation of Wilms' tumor gene (wt1) during myelomonocytic differentiation in HL60 cells. *Blood*, 83(7), 1876-82 (1994)

107.Q. Wang, H. Salman, M. Danilenko and G. P. Studzinski: 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*, 204(3), 964-74 (2005)

108.G. Vereb, Jr., J. Szollosi, L. Matyus, M. Balazs, W. C. Hyun and B. G. Feuerstein: Depletion of intracellular calcium stores facilitates the influx of extracellular calcium in platelet derived growth factor stimulated A172 glioblastoma cells. *Cytometry*, 24(1), 64-73 (1996)

109.G. G. Kovacs, A. Zsembery, S. J. Anderson, P. Komlosi, G. Y. Gillespie, P. D. Bell, D. J. Benos and C. M. Fuller: Changes in intracellular Ca2+ and pH in response to thapsigargin in human glioblastoma cells and normal astrocytes. *Am J Physiol Cell Physiol*, 289(2), C361-71 (2005)

110.S. Chigurupati, R. Venkataraman, D. Barrera, A. Naganathan, M. Madan, L. Paul, J. V. Pattisapu, G. A. Kyriazis, K. Sugaya, S. Bushnev, J. D. Lathia, J. N. Rich and S. L. Chan: Receptor channel TRPC6 is a key mediator of Notch-driven glioblastoma growth and invasiveness. *Cancer Res*, 70(1), 418-27 (2010)

111.X. Ding, Z. He, K. Zhou, J. Cheng, H. Yao, D. Lu, R. Cai, Y. Jin, B. Dong, Y. Xu and Y. Wang: Essential Role of TRPC6 Channels in G2/M Phase Transition and Development of Human Glioma. *J Natl Cancer Inst* (2010)

112.H. Wu, J. M. Yang, S. Jin, H. Zhang and W. N. Hait: Elongation factor-2 kinase regulates autophagy in human glioblastoma cells. *Cancer Res*, 66(6), 3015-23 (2006)

113.T. Szado, V. Vanderheyden, J. B. Parys, H. De Smedt, K. Rietdorf, L. Kotelevets, E. Chastre, F. Khan, U. Landegren, O. Soderberg, M. D. Bootman and H. L. Roderick: Phosphorylation of inositol 1,4,5-trisphosphate receptors by protein kinase B/Akt inhibits Ca2+ release and apoptosis. *Proc Natl Acad Sci U S A*, 105(7), 2427-32 (2008)

114.A. Yacoub, H. A. Hamed, J. Allegood, C. Mitchell, S. Spiegel, M. S. Lesniak, B. Ogretmen, R. Dash, D. Sarkar, W. C. Broaddus, S. Grant, D. T. Curiel, P. B. Fisher and P. Dent: 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*, 70(3), 1120-9 (2010)

115.C. A. Scrideli, C. G. Carlotti, Jr., O. K. Okamoto, V. S. Andrade, M. A. Cortez, F. J. Motta, A. K. Lucio-Eterovic, L. Neder, S. Rosemberg, S. M. Oba-Shinjo, S. K. Marie and L. G. Tone: 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*, 88(3), 281-91 (2008)

116.Y. Ruano, M. Mollejo, T. Ribalta, C. Fiano, F. I. Camacho, E. Gomez, A. R. de Lope, J. L. Hernandez-Moneo, P. Martinez and B. Melendez: Identification of novel candidate target genes in amplicons of Glioblastoma multiforme tumors detected by expression and CGH microarray profiling. *Mol Cancer*, 5, 39 (2006)

117.C. Liu, J. Yao, D. Mercola and E. Adamson: The transcription factor EGR-1 directly transactivates the fibronectin gene and enhances attachment of human glioblastoma cell line U251. *J Biol Chem*, 275(27), 20315-23 (2000)

118.R. P. Huang, C. Liu, Y. Fan, D. Mercola and E. D. Adamson: Egr-1 negatively regulates human tumor cell growth via the DNA-binding domain. *Cancer Res*, 55(21), 5054-62 (1995)

119.M. Mittelbronn, P. Harter, A. Warth, A. Lupescu, K. Schilbach, H. Vollmann, D. Capper, B. Goeppert, K. Frei, H. Bertalanffy, M. Weller, R. Meyermann, F. Lang and P. Simon: EGR-1 is regulated by N-methyl-D-aspartate-receptor stimulation and associated with patient survival in human high grade astrocytomas. *Brain Pathol*, 19(2), 195-204 (2009)

120.A. J. Clark, J. L. Ware, M. Y. Chen, M. R. Graf, T. E. Van Meter, W. G. Dos Santos, H. L. Fillmore and W. C.

Broaddus: Effect of WT1 gene silencing on the tumorigenicity of human glioblastoma multiforme cells. *J Neurosurg*, 112(1), 18-25 (2010)

121.N. Hashimoto, A. Tsuboi, Y. Chiba, S. Izumoto, Y. Oka, T. Yoshimine and H. Sugiyama: [Immunotherapy targeting the Wilms' tumor 1 gene product for patients with malignant brain tumors]. *Brain Nerve*, 61(7), 805-14 (2009)

122.Y. Nakahara, H. Okamoto, T. Mineta and K. Tabuchi: Expression of the Wilms' tumor gene product WT1 in glioblastomas and medulloblastomas. *Brain Tumor Pathol*, 21(3), 113-6 (2004)

123.J. Schittenhelm, M. Mittelbronn, T. D. Nguyen, R. Meyermann and R. Beschorner: WT1 expression distinguishes astrocytic tumor cells from normal and reactive astrocytes. *Brain Pathol*, 18(3), 344-53 (2008)

124.T. Hashiba, S. Izumoto, N. Kagawa, T. Suzuki, N. Hashimoto, M. Maruno and T. Yoshimine: Expression of WT1 protein and correlation with cellular proliferation in glial tumors. *Neurol Med Chir (Tokyo)*, 47(4), 165-70; discussion 170 (2007)

125.M. Flourakis and N. Prevarskaya: Insights into Ca^{2+} homeostasis of advanced prostate cancer cells. *Biochim Biophys Acta*, 1793(6), 1105-9 (2009)

126.Y. Gong, L. J. Blok, J. E. Perry, J. K. Lindzey and D. J. Tindall: Calcium regulation of androgen receptor expression in the human prostate cancer cell line LNCaP. *Endocrinology*, 136(5), 2172-8 (1995)

127.K. Vanoverberghe, F. Vanden Abeele, P. Mariot, G. Lepage, M. Roudbaraki, J. L. Bonnal, B. Mauroy, Y. Shuba, R. Skryma and N. Prevarskaya: Ca^{2+} homeostasis and apoptotic resistance of neuroendocrine-differentiated prostate cancer cells. *Cell Death Differ*, 11(3), 321-30 (2004)

128.F. Vanden Abeele, R. Skryma, Y. Shuba, F. Van Coppenolle, C. Slomianny, M. Roudbaraki, B. Mauroy, F. Wuytack and N. Prevarskaya: Bcl-2-dependent modulation of Ca(2+) homeostasis and store-operated channels in prostate cancer cells. *Cancer Cell*, 1(2), 169-79 (2002)

129.J. Yu, I. de Belle, H. Liang and E. D. Adamson: Coactivating factors p300 and CBP are transcriptionally crossregulated by Egr1 in prostate cells, leading to divergent responses. *Mol Cell*, 15(1), 83-94 (2004)

130.S. A. Abdulkadir, J. M. Carbone, C. K. Naughton, P. A. Humphrey, W. J. Catalona and J. Milbrandt: Frequent and early loss of the EGR1 corepressor NAB2 in human prostate carcinoma. *Hum Pathol*, 32(9), 935-9 (2001)

131.J. Svaren, T. Ehrig, S. A. Abdulkadir, M. U. Ehrengruber, M. A. Watson and J. Milbrandt: EGR1 target genes in prostate carcinoma cells identified by microarray analysis. *J Biol Chem*, 275(49), 38524-31 (2000)

132.Y. Furuya, P. Lundmo, A. D. Short, D. L. Gill and J. T. Isaacs: The role of calcium, pH, and cell proliferation in the programmed (apoptotic) death of androgen-independent prostatic cancer cells induced by thapsigargin. *Cancer Res*, 54(23), 6167-75 (1994)

133.S. B. Christensen, A. Andersen, H. Kromann, M. Treiman, B. Tombal, S. Denmeade and J. T. Isaacs: Thapsigargin analogues for targeting programmed death of androgen-independent prostate cancer cells. *Bioorg Med Chem*, 7(7), 1273-80 (1999)

134.H. Sohoel, A. M. Jensen, J. V. Moller, P. Nissen, S. R. Denmeade, J. T. Isaacs, C. E. Olsen and S. B. Christensen: Natural products as starting materials for development of second-generation SERCA inhibitors targeted towards prostate cancer cells. *Bioorg Med Chem*, 14(8), 2810-5 (2006)

135.C. Picard, C. A. McCarl, A. Papolos, S. Khalil, K. Luthy, C. Hivroz, F. LeDeist, F. Rieux-Laucat, G. Rechavi, A. Rao, A. Fischer and S. Feske: STIM1 mutation associated with a syndrome of immunodeficiency and autoimmunity. *N Engl J Med*, 360(19), 1971-80 (2009)

136.R. T. Greenlee, M. B. Hill-Harmon, T. Murray and M. Thun: Cancer statistics, 2001. *CA Cancer J Clin*, 51(1), 15-36 (2001)

137.N. Auersperg, A. S. Wong, K. C. Choi, S. K. Kang and P. C. Leung: Ovarian surface epithelium: biology, endocrinology, and pathology. *Endocr Rev*, 22(2), 255-88 (2001)

138.C. M. Salamanca, S. L. Maines-Bandiera, P. C. Leung, Y. L. Hu and N. Auersperg: Effects of epidermal growth factor/hydrocortisone on the growth and differentiation of human ovarian surface epithelium. *J Soc Gynecol Investig*, 11(4), 241-51 (2004)

139.P. Hohenstein and N. D. Hastie: The many facets of the Wilms' tumour gene, WT1. *Hum Mol Genet*, 15 Spec No 2, R196-201 (2006)

140.S. Hosono, X. Luo, D. P. Hyink, L. M. Schnapp, P. D. Wilson, C. R. Burrow, J. C. Reddy, G. F. Atweh and J. D. Licht: WT1 expression induces features of renal epithelial differentiation in mesenchymal fibroblasts. *Oncogene*, 18(2), 417-27 (1999)

141.L. Saxen and H. Sariola: Early organogenesis of the kidney. *Pediatr Nephrol*, 1(3), 385-92 (1987)

142.K. Pritchard-Jones, S. Fleming, D. Davidson, W. Bickmore, D. Porteous, C. Gosden, J. Bard, A. Buckler, J. Pelletier, D. Housman and *et al.*: The candidate Wilms' tumour gene is involved in genitourinary development. *Nature*, 346(6280), 194-7 (1990)

143.J. A. Kreidberg, H. Sariola, J. M. Loring, M. Maeda, J. Pelletier, D. Housman and R. Jaenisch: WT-1 is required for early kidney development. *Cell*, 74(4), 679-91 (1993)

144.M. Shimizu, T. Toki, Y. Takagi, I. Konishi and S. Fujii: Immunohistochemical detection of the Wilms' tumor gene (WT1) in epithelial ovarian tumors. *Int J Gynecol Pathol*, 19(2), 158-63 (2000)

145.S. Yamamoto, H. Tsuda, T. Kita, K. Maekawa, K. Fujii, K. Kudoh, K. Furuya, S. Tamai, J. Inazawa and O. Matsubara: Clinicopathological significance of WT1 expression in ovarian cancer: a possible accelerator of tumor progression in serous adenocarcinoma. *Virchows Arch*, 451(1), 27-35 (2007)

146.M. V. Barbolina, B. P. Adley, L. D. Shea and M. S. Stack: Wilms tumor gene protein 1 is associated with ovarian cancer metastasis and modulates cell invasion. *Cancer*, 112(7), 1632-41 (2008)

147.W. Netinatsunthorn, J. Hanprasertpong, C. Dechsukhum, R. Leetanaporn and A. Geater: WT1 gene expression as a prognostic marker in advanced serous epithelial ovarian carcinoma: an immunohistochemical study. *BMC Cancer*, 6, 90 (2006)

148.B. Hylander, E. Repasky, P. Shrikant, M. Intengan, A. Beck, D. Driscoll, P. Singhal, S. Lele and K. Odunsi: Expression of Wilms tumor gene (WT1) in epithelial ovarian cancer. *Gynecol Oncol*, 101(1), 12-7 (2006)

149.S. M. Huber, G. S. Braun, S. Segerer, R. W. Veh and M. F. Horster: Metanephrogenic mesenchyme-to-epithelium transition induces profound expression changes of ion channels. *Am J Physiol Renal Physiol*, 279(1), F65-76 (2000)

150.P. J. Cullen and P. J. Lockyer: Integration of calcium and Ras signalling. *Nat Rev Mol Cell Biol*, 3(5), 339-48 (2002)

151.C. R. Schmidt, M. K. Washington, Y. J. Gi, R. J. Coffey, R. D. Beauchamp and A. S. Pearson: Dysregulation of E-cadherin by oncogenic Ras in intestinal epithelial cells is blocked by inhibiting MAP kinase. *Am J Surg*, 186(5), 426-30 (2003)

152.G. Christofori and H. Semb: The role of the cell-adhesion molecule E-cadherin as a tumour-suppressor gene. *Trends Biochem Sci*, 24(2), 73-6 (1999)

153.K. Sawada, A. K. Mitra, A. R. Radjabi, V. Bhaskar, E. O. Kistner, M. Tretiakova, S. Jagadeeswaran, A. Montag, A. Becker, H. A. Kenny, M. E. Peter, V. Ramakrishnan, S. D. Yamada and E. Lengyel: Loss of E-cadherin promotes ovarian cancer metastasis via alpha 5-integrin, which is a therapeutic target. *Cancer Res*, 68(7), 2329-39 (2008)

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