Voltage-gated calcium channels: Novel targets for cancer therapy

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Abstract. Voltage-gated calcium channels (VGCCs) comprise five subtypes: The L-type; R-type; N-type; P/Q-type; and T-type, which are encoded by α_1 subunit genes. Calcium ion channels also have confirmed roles in cellular functions, including mitogenesis, proliferation, differentiation, apoptosis and metastasis. An association between VGCCs, a reduction in proliferation and an increase in apoptosis in prostate cancer cells has also been reported. Therefore, in the present study, the online clinical database Oncomine was used to identify the alterations in the mRNA expression level of VGCCs in 19 cancer subtypes. Overall, VGCC family genes exhibited under-expression in numerous types of cancer, including brain, breast, kidney and lung cancers. Notably, the majority of VGCC family members (CACNA1C, CACNA1D, CACNA1A, CACNA1B, CACNA1E, CACNA1H and CACNA1I) exhibited low expression in brain tumors, with mRNA expression levels in the top 1-9% of downregulated gene rankings. A total of 5 VGCC family members (CACNA1A, CACNA1B, CACNA1E, CACNA1G and CACNA1I) were under-expressed in breast cancer, with a gene ranking in the top 1-10% of the low-expressed genes compared with normal tissue. In kidney and lung cancers, CACNA1S, CACNA1C, CACNA1D, CACNA1A and CACNA1H exhibited low expression, with gene rankings in the top 1-8% of downregulated genes. In conclusion, the present findings may contribute to the development of new cancer treatment approaches by identifying target genes involved in specific types of cancer.

Introduction

Conventional studies on ion channels have primarily focused on the crucial roles these channels perform in excitatory cell types, including neurons, cardiomyocytes and secretory cells (1). The roles of ion channels in various cell functions, including mitogenesis, cell proliferation, differentiation, apoptosis and metastasis are now well recognized (2-4). The expression of ion channel transcripts has been highlighted as a potential biomarker of certain types of cancer, including prostate cancer and breast cancer (5-8). Therefore, the investigation of the functional roles of ion channels in cancer development may identify novel approaches for tumor prognosis (9,10).

Calcium channels may be generally categorized into two major classes: Voltage-gated calcium channels (VGCCs) and ligand-gated calcium channels (LGCCs). VGCCs may be classified into five subtypes: L-type (10,11); N-Type (12); P-type (13-15); T-type (16-18); and R-type (19,20). These ion channels have been implicated in the progression of numerous cancers. Members of the LGCC class, namely the inositol trisphosphate receptor (21) and ryanodine (22), are also well documented to regulate certain processes occurring during cancer metastasis.

The role of calcium has been well documented in numerous cellular processes, including cell proliferation and inhibition and activation of various intracellular enzymes (23-25). The effect of calcium on these processes varies by location, extent and calcium homeostasis stage (26,27). The transcript levels of calcium channels may cause different domino effects on specific cell functions, as well as on cell proliferation, motility or even cell apoptosis (28).

The recessive inheritance pattern of tumor suppressor genes presents the greatest challenge in their identification. Another challenge is the diversity of their action mechanisms, which

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range from variations in structure and copy number to epigenetic changes and single base modifications. In addition, the molecular mechanism of action of a tumor suppressor gene may change depending on the tumor type. These variations affect the abnormal activation of oncogenes and/or the inactivation of tumor suppressor genes, resulting in tumor formation and development.

Expression levels of genes are currently recognized as potential biological markers (29-32). The recent publication of a public database (Oncomine; www.oncomine.org) containing mRNA expression profiles for various cancers has now led to the supply of a prodigious number of datasets, which may improve the identification of critical biomarkers in cancers and assist the development of improved molecular signatures, once in full operation (33,34). However, tumor suppressor genes tend to exhibit low or reduced expression in tumor tissue compared with normal tissue. A previous study specifically evaluated a tumor suppressor gene in breast cancer datasets from the Oncomine database and observed significant down-regulation and low expression of the tumor suppressor gene ADAM metallopeptidase with thrombospondin type 1 motif 1 in breast carcinomas, when compared with normal tissue (35). Another study revealed that the expression of sirtuin-3 (SIRT3), a mitochondria-localized tumor suppressor, was decreased in breast tumors relative to normal type-matched tissue. SIRT3 induced destabilization of hypoxia-inducible factor-1α, whereas knockdown of SIRT3 increased cancer cell growth and angiogenesis (36). The SIRT3 gene also exhibited lower expression in various tumor types, including clear cell hepatocellular, head and neck squamous cell, glioblastoma multiforme, testicular and prostate cancers (37).

VGCCs are likely to be possible targets for future clinical cancer treatments. The blockage of T-type calcium channels in HCT116 cells was shown to inhibit cell development and promote apoptosis (38). The T-type calcium channel also confers anti-proliferative properties in malignant tumor cells (39). Cells that highly express the majority of types of calcium channels are significantly reduced in their proliferation capabilities (40). The calcium voltage-gated channel subunit α1 G (CACNA1G; Cav3.1) gene, a T-type α subunit of a calcium channel, has been studied in colorectal cancer for its involvement in methylation and silencing. CACNA1G is also considered a potential tumor suppressor gene, which may reduce the effects of hypermethylation of CpG islands, thereby attenuating cancer development (41). Another study reported an association between the CACNA1G gene, a reduction in proliferation and an increase in apoptosis in prostate cancer cells. However, the molecular mechanisms of the role of calcium channels as tumor suppressors remain unclear and require additional study (42).

The authors of the present study previously performed a meta-analysis on public microarray datasets and demonstrated the existence of voltage-gated calcium gene signatures in patients with cancer (43). However, compared with normal tissue, certain VGCC family genes exhibited low expression in tumor tissue. In addition, at present, the way in which VGCC family genes function as tumor suppressors in cancer development remains largely unknown. To the best of our knowledge, the present study is the first exploration of the reduced expression of VGCC genes in cancer development, and the present findings indicated that VGCC genes may be prospective targets for cancer treatment. Based on bioinformatics screening, the under-expression of VGCCs at the mRNA level has raised the question of their possible association with the development and progression of cancer. This potential association between VGCCs and cancer was investigated using Oncomine, a clinical online microarray database (44). The aim of the present study was to examine the expression of VGCC genes in various types and subtypes of cancer, and to identify critical indicators supporting prospective research on the role of VGCC channels in cancer onset and progression.

Materials and methods

Meta-analysis. According to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis guidelines, a meta-analysis of public microarray data was performed to analyze the under-expression of VGCC mRNA in clinical cancer tissue (Fig. 1) (45,46). Oncomine (www.oncomine.org) was used to conduct a systematic analysis of all public cancer microarray data (44). The Oncomine Platform Overview Q1 2014 website comprises >700 independent datasets with ~90,000 microarray experiments. Analyses of these data determined the under-expression of VGCC genes in diverse types and subtypes of cancer.

Threshold specifications were set to evaluate the potential tumor suppressor genes in the datasets that could control VGCC transcript expression in cancer tissue. Samples that showed under-expression with a fold change <-2.0, P<0.001 and a gene rank in the top 10% were included in the analysis. A fold-change-based standard for under-expression was used to establish the linear model association between mRNA levels and VGCC gene expression in cancer tissues compared with normal expression levels in matched tissue. The P-values were used to determine the gene rank percentile, indicating the degree of expression. Ultimately, 34 studies were retained, comprising 4,443 samples (47,48). To investigate VGCC expression in cancer cell lines, gene expression data on VGCC genes across a panel of 967 cancer cell lines was downloaded and analyzed from the Cancer Cell Line Encyclopedia (CCLE) (49).

Results and Discussion

Voltage-gated calcium channel family in cancer development. VGCCs consist of the α1 subunit, which controls the construction of calcium selective pores. Five subtypes of VGCC are recognized, consisting of the L-type, R-type, N-type, P/Q-type and T-type, which are encoded by the α1 subunit genes (50,51). CACNA1S, CACNA1C, CACNA1D and CACNA1F encode the L-type, CACNA1E encodes the R-type, CACNA1B encodes the N-type, CACNA1A encodes the P/Q type, and CACNA1G, CACNA1H and CACNA1I encode the T-type (52). Tumor suppressor genes have been well documented as cell division regulators that control protein expression. A mutation of these anti-oncogenes may affect cell proliferation, cell cycle and apoptosis, which is then likely to trigger the onset of a specific type of cancer (53). In cancer tissues, abnormal cell growth is mainly caused by the inactivation or lack of tumor suppressor genes (54). Tumor suppressor genes are also a type of cell
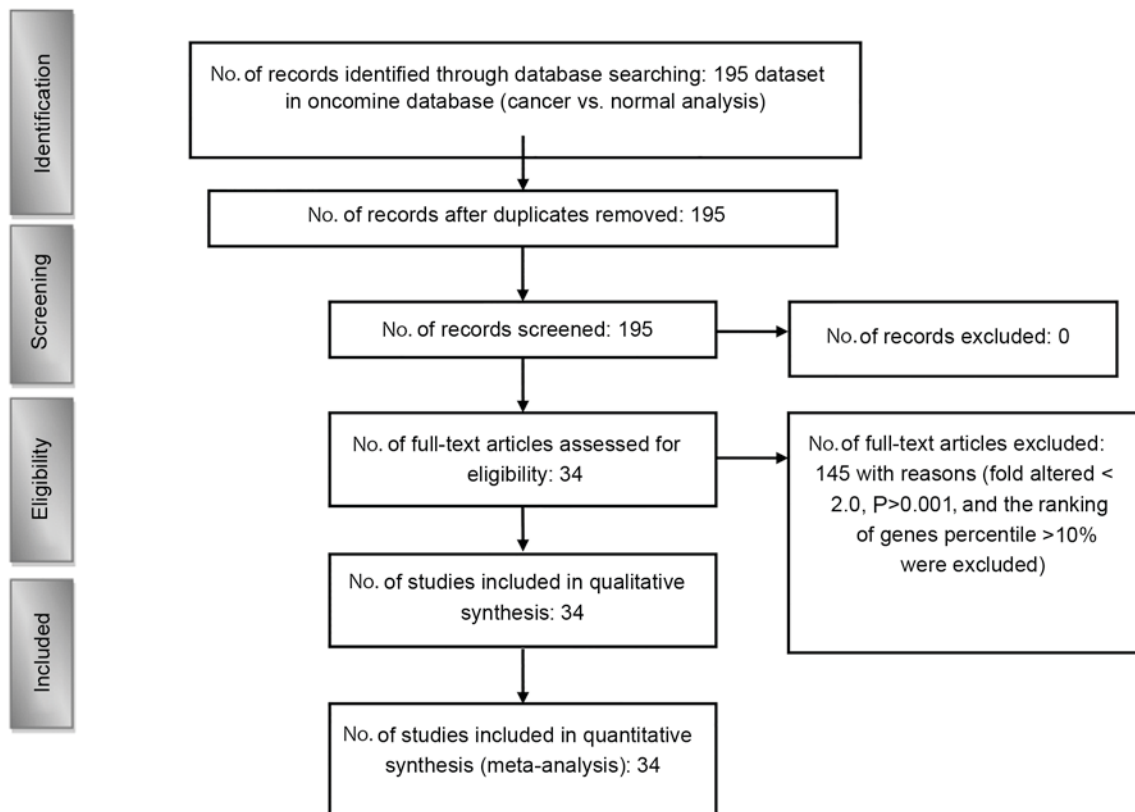
Figure 1. Flow chart describing the process of data identification and accumulation for the quantitative analysis.

growth inhibitor and suppress tumor formation genes. Tumor suppressor genes are also under-expressed or downregulated in tumor tissues compared with normal tissue (53).

Previous studies have specifically used the Oncomine database to screen and evaluate tumor suppressor genes. For example, protein tyrosine phosphatase, receptor type D was expressed at significantly lower levels in glioma relative to matched tissue in the brain (55). Another study using the Oncomine database revealed that the level of castor zinc finger 1 (CASZ1) expression was lower in numerous cancer cell types, including gastric intestinal-type adenocarcinoma, head and neck squamous cell carcinoma and clear cell renal cell carcinoma. CASZ1 was also revealed to function as a tumor-suppressor in neuroblastoma (56). A high expression of CASZ1 may interrupt the cancer development process of other types of tissue and play a role in their tumorigenesis (57).

The present bioinformatics analysis revealed that CACNA1S, CACNA1C, CACNA1D and CACNA1A were abundantly expressed in normal tissue but not in cancer tissue; thus, they could serve as tumor suppressor gene markers for specific subtypes of cancer. For these specific types and subtypes of cancer, patients may be supplemented with healthy food or a calcium agonist. This would enable the regulation of CACNA1S, CACNA1C, CACNA1D and CACNA1A signal transduction, thereby offering a method to treat cancer and even cardiovascular disease (58-61), obesity (62) and diabetes (63-65). However, no global method currently exists to screen for the under-expression of VGCCs in diverse types and subtypes of cancers. Therefore, the present study used Oncomine, an online clinical microarray database, to calculate

the changes in mRNA expression levels of VGCCs in 20 types of cancer, and to determine a suitable threshold based on P-values (<0.001), fold change (<-2.0) and gene rank (<top 10%) to select satisfactory datasets. Fig. 1 showed VGCC gene under-expression in 20 cancers, including the corresponding fold changes, P-values and top gene ranks.

A bioinformatics approach was performed in the current study to determine the functions of VGCC genes in the development of cancer, and to determine whether VGCC genes are potential tumor suppressor genes similar to PTEN (66) or SIRT3. The VGCC genes showed downregulation in 18 of the 20 examined cancer tissues compared with normal tissue (Fig. 1). Overall, VGCC family genes showed under-expression in numerous types of cancers, including brain, breast, kidney and lung cancers. Notably, when compared with their expression in normal tissue, the majority of VGCC family members (CACNA1C, CACNA1D, CACNA1A, CACNA1B, CACNA1E, CACNA1H and CACNA1I) were found to be downregulated in brain tumors, with an mRNA expression level in the top 1-9% of the gene rankings. In total, 5 of the VGCC family members (CACNA1A, CACNA1B, CACNA1E, CACNA1G and CACNA1I) were under-expressed in breast cancer, with a gene ranking in the top 1-10% of the expressed genes relative to normal tissue. In kidney cancer and lung cancer, CACNA1S, CACNA1C, CACNA1D, CACNA1A and CACNA1H had low levels of expression, with a gene ranking in the top 1-8% of the expressed genes. In addition, certain VGCC family genes were under-expressed in other cancer subtypes, including colorectal, lymphoma, ovarian and bladder cancers.

	CACNA1S	CACNA1C	CACNA1D	CACNA1F	CACNA1A	CACNA1B	CACNA1E	CACNA1G	CACNA1H	CACNA1I
Bladder		6						4	2	
Brain and CNS		1	6		1	1	3	10	1	2
Breast					1	5	5	3	5	
Cervical		10								
Colorectal		10			8			8		
Esophageal					1					
Gastric					3		1			
Head and neck	4									
Kidney	3									
Myeloma			3							
Leukemia							5			
Liver										
Lung	7	1	1				7		5	
Lymphoma		2		1						
Ovarian								5	7	
Pancreatic										
Prostate		2					1			
Renal		2	1					3		
Sacroma			1							

10	5	1

Gene rank percentile

Figure 2. Under-expression of 10 voltage-gated calcium channel genes in 19 types of cancers. The comparison of cancer vs. normal tissue was shown at different expression levels based on P<0.001, a fold change >2.0 and a gene rank percentile <10%, to screen the microarray datasets. The color alterations represent the changes in level of gene rank percentage.

L-type calcium channel family. The L-type calcium channel is encoded by four genes: Cav1.1 (CACNA1S); Cav1.2 (CACNA1C); Cav1.3 (CACNA1D); and Cav1.4 (CACNA1F). These genes are generally located in different areas, including skeletal muscle, smooth muscle, bone (osteoblasts) and ventricular myocytes. Previous studies explored the role of L-type calcium channels primarily in the physiology and pharmacology fields (67,68). Therefore, their functions are largely unknown in terms of their role in cancer progression. Notably, compared with our previous studies, CACNA1S was overexpressed relative to normal tissue samples in acute myeloid leukemia and brain and central nervous system (CNS) tumors (1). The present data revealed downregulated CACNA1S in renal oncocytoma, with a -3.920-fold change, as well as in head and neck squamous cell carcinoma, (3.670-fold change) and in squamous cell lung carcinoma (-2.038-fold change), compared with matched normal tissue (Table I). In addition, CACNA1S ranked in the top 5% of downregulated genes in head-neck and renal cancer (Fig. 2). Thus, CACNA1S showed increased mRNA expression in a number of cancer tissues but decreased expression in others. These findings indicated that cell context-specific alterations in CACNA1S expression may play a critical role in cancer biology.

CACNA1C has been revealed to affect the pathophysiology of psychiatric disease (69). The present bioinformatics analysis confirmed that CACNA1C exhibited low expressions in the majority of types of cancer, including brain, lymphoma, ovarian, bladder, prostate, renal, salivary gland, cervix and colorectal cancers, compared with normal tissue (Table I). In addition, 9 of the 20 types of cancer showed downregulation in which CACNA1C was ranked in the top 5% of genes that had low expression (Fig. 2). Compared with normal tissue,

CACNA1C was significantly downregulated in the majority of brain tumor types, including diffuse astrocytoma, glioblastoma, anaplastic astrocytoma and oligodendroglia, with P-values ranging between 1.77×10^{-4} and 9.26×10^{-21} and a gene ranking between 1 and 6%. Lastly, CACNA1C expression decreased in centroblastic lymphoma, with a -2.381-fold increase, a P-value of 3.77×10^{-18} and a gene ranking in the top 1% relative to matched normal tissue sections. Compared with our previous study (43), CACNA1C was overexpressed compared with normal tissue samples in gastric, pancreas, brain, colorectal, breast, uterus, skin and prostate cancers and leukemia. Notably, up- and downregulated CACNA1C was observed in brain, prostate and colorectal tumors compared with normal tissue. The conflicting expression profiles of CACNA1C in the same types of cancer may be due to the broad range of categories for each cancer subtype. For example, our previous study (43) indicated that CACNA1C had high expression in prostate carcinoma; however, in the present study, CACNA1C had low expression in prostatic intraepithelial neoplasia epithelia relative to normal type-matched tissue. In addition, CACNA1C had high expression in colon and renal adenoma; however, in the present study, CACNA1C had low expression in rectosigmoid adenocarcinoma relative to normal matched tissue. In addition, CACNA1C was up- and downregulated in brain glioblastoma. The small sample size in the original publications (70,71) may explain this discrepancy. Collectively, our data suggested that alterations in CACNA1C expression may have an adverse effect on tissue homeostasis, which may result in tumorigenesis.

CACNA1D regulates cell firing (21) and is associated with prostate cancer; however, the presence of CACNA1D in other cancer subtypes requires confirmation. The present

Table I. L-type calcium channel expression in cancers.

Gene and cancer type	Patient number, n	P-value (cancer/normal)	t-test (cancer/normal)	Fold (cancer/normal)	Gene ranking, %	Database reference	(Refs.)
CACNA1S							
Renal							
Renal oncocytoma	92	1.77×10^{-16}	-26.050	-3.920	303 (top 3)	Clin Cancer Res 2005/08/15	(94)
Papillary renal cell carcinoma	92	4.88×10^{-15}	-21.978	-2.934	324 (top 3)	Clin Cancer Res 2005/08/15	(94)
Head-neck							
Head and neck squamous cell carcinoma	54	6.96×10^{-9}	-7.318	-3.670	748 (top 6)	Cancer Res 2004/01/01	(95)
Tongue squamous cell carcinoma	58	5.26×10^{-6}	-5.261	-6.638	324 (top 4)	BMC Cancer 2009/01/12	(96)
Lung							
Squamous cell lung carcinoma	93	8.64×10^{-7}	-6.038	-2.038	601 (top 7)	Cancer Res 2005/04/15	(97)
CACNA1C							
Brain							
Glioblastoma	180	9.26×10^{-21}	-14.125	-4.483	484 (top 3)	Cancer Cell 2006/04/01	(70)
Oligodendroglioma	180	1.39×10^{-14}	-10.049	-3.024	304 (top 2)	Cancer Cell 2006/04/01	(70)
Anaplastic astrocytoma	180	7.46×10^{-11}	-8.854	-3.166	72 (top 1)	Cancer Cell 2006/04/01	(70)
Diffuse astrocytoma	180	4.59×10^{-4}	-4.878	-2.505	619 (top 4)	Cancer Cell 2006/04/01	(70)
Glioblastoma	84	1.77×10^{-4}	-11.513	-2.550	1,173 (top 6)	J Clin Oncol 2008/06/20	(71)
Lymphoma							
Centroblastic lymphoma	336	3.77×10^{-18}	-13.615	-2.381	17 (top 1)	Nat Genet 2005/04/01	(98)
Ovarian							
Ovarian serous adenocarcinoma	53	1.72×10^{-14}	-13.253	-27.228	218 (top 2)	Cancer Sci 2009/08/01	(99)
Bladder							
Infiltrating bladder urothelial carcinoma	157	9.94×10^{-12}	-7.365	-3.364	675 (top 6)	J Clin Oncol 2006/02/10	(100)
Superficial bladder cancer	157	2.00×10^{-10}	-8.288	-6.787	750 (top 6)	J Clin Oncol 2006/02/10	(100)
Prostate							
Prostatic intraepithelial neoplasia epithelia	101	2.80×10^{-5}	-4.635	-3.139	134 (top 2)	Nat Genet 2007/01/01	(101)
Prostate carcinoma epithelia	101	7.53×10^{-4}	-3.381	-2.192	524 (top 5)	Nat Genet 2007/01/01	(101)
Renal							
Papillary renal cell carcinoma	67	4.95×10^{-6}	-5.711	-2.297	308 (top 2)	BMC Cancer 2009/05/18	(102)
Salivary gland							
Salivary gland adenoid cystic carcinoma	22	5.15×10^{-6}	-5.962	-22.108	257 top 3)	Am J Pathol 2002/10/01	(103)
Cervix							
Cervical squamous cell carcinoma	45	1.25×10^{-4}	-8.154	-2.377	1,775 (top 10)	Gynecol Oncol 2008/03/01	(104)
Colorectal							

Table I. Continued.

Gene and cancer type	Patient number, n	P-value (cancer/normal)	t-test (cancer/normal)	Fold (cancer/normal)	Gene ranking, %	Database reference	(Refs.)
Rectosigmoid adenocarcinoma	237	5.06×10^{-4}	-3.880	-2.273	1861 (top 10)	TCGA 2011/09/08	
CACNA1D							
Lung							
Squamous cell lung carcinoma	156	7.16×10^{-31}	-19.447	-4.801	51 (top 1)	PLoS One 2010/04/22	(105)
Lung adenocarcinoma	246	2.15×10^{-24}	-13.057	-2.443	45 (top 1)	Cancer Res 2012/01/01	(106)
Brain							
Glioblastoma	180	3.23×10^{-16}	-10.515	-2.850	1,122 (top 6)	Cancer Cell 2006/04/01	(70)
Anaplastic astrocytoma	180	3.49×10^{-6}	-5.415	-2.094	1,722 (top 9)	Cancer Cell 2006/04/01	(70)
Diffuse astrocytoma	180	0.001	-4.432	-2.814	1,117 (top 6)	Cancer Cell 2006/04/01	(70)
Myeloma							
Monoclonal gammopathy of undetermined significance	78	3.65×10^{-6}	-4.889	-2.563	435 (top 3)	Blood 2007/02/15	(107)
Smoldering myeloma	78	2.30×10^{-4}	-4.507	-3.487	552 (top 3)	Blood 2007/02/15	(107)
Sarcoma							
Dedifferentiated liposarcoma	54	2.88×10^{-4}	-4.694	-4.980	102 (top 1)	Cancer Res 2005/07/01	(108)
Malignant fibrous histiocytoma	54	6.13×10^{-4}	-3.790	-4.255	514 (top 5)	Cancer Res 2005/07/01	(108)
Renal							
Renal Wilms tumor	67	3.71×10^{-4}	-6.265	-2.958	144 (top 1)	BMC Cancer 2009/05/18	(102)
CACNA1F							
Lymphoma							
Anaplastic large cell lymphoma	60	1.14×10^{-12}	-13.057	-2.044	43 (top 1)	J Clin Invest 2007/03/01	(73)

Table II. P-type calcium channel expression in cancers.

Gene and cancer type	Patient number, n	P-value (cancer/normal)	t-test (cancer/normal)	Fold (cancer/normal)	Gene ranking, %	Database reference	(Refs.)
CACNA1A							
Brain							
Glioblastoma	180	8.77×10^{-18}	-10.656	-3.039	841 (top 5)	Cancer Cell 2006/04/01	(70)
Anaplastic astrocytoma	180	3.02×10^{-8}	-6.935	-2.188	637 (top 4)	Cancer Cell 2006/04/01	(70)
Brain glioblastoma	557	2.09×10^{-13}	-24.201	-6.900	330 (top 3)	TCGA 2013/06/03	
Glioblastoma	84	4.22×10^{-6}	-8.968	-2.424	585 (top 3)	J Clin Oncol 2008/06/20	(71)
Astrocytoma	51	2.54×10^{-5}	-5.241	-7.344	148 (top 3)	Cancer Res 2001/09/15	(109)
Atypical teratoid/rhabdoid tumor	85	9.05×10^{-5}	-7.761	-523.335	24 (top 1)	Nature 2002/01/24	(11)
Malignant glioma, NOS	85	2.07×10^{-4}	-5.012	-139.970	80 (top 2)	Nature 2002/01/24	(11)
Primitive neuroectodermal tumor, NOS	85	4.64×10^{-4}	-10.153	-704.199	34 (top 1)	Nature 2002/01/24	(11)
Classic medulloblastoma	85	5.73×10^{-4}	-5.011	-51.831	411 (top 8)	Nature 2002/01/24	(11)
Desmoplastic medulloblastoma	85	8.98×10^{-4}	-3.937	-49.283	199 (top 4)	Nature 2002/01/24	(11)
Oligodendroglioma	42	1.09×10^{-4}	-6.523	-2.168	289 (top 4)	Oncogene 2003/07/31	(110)
Astrocytoma	42	2.84×10^{-4}	-5.138	-2.084	141 (top 2)	Oncogene 2003/07/31	(110)
Colorectal							
Colorectal carcinoma	82	5.25×10^{-6}	-5.293	-2.807	1,830 (top 10)	Clin Exp Metastasis 2010/02/01	(111)
Colon adenoma	64	3.39×10^{-5}	-4.402	-3.376	1,451 (top 8)	Mol Cancer Res 2007/12/01	(112)
Esophagus							
Esophagus	48	9.61×10^{-5}	-5.368	-2.873	146 (top 1)	Gastroenterology 2006/09/01	(20)
Gastric							
Gastric intestinal type adenocarcinoma	69	2.25×10^{-4}	-3.737	-2.370	1,040 (top 6)	Eur J Cancer 2009/02/01	(113)
Gastric mixed adenocarcinoma	69	3.40×10^{-4}	-5.809	-6.673	426 (top 3)	Eur J Cancer 2009/02/01	(113)
Breast							
Invasive ductal breast carcinoma	30	9.34×10^{-4}	-3.536	-2.205	189 (top 1)	BMC Cancer 2007/03/27	(114)

NOS, not otherwise specified.

study revealed that CACNA1D expression levels decreased in lung, brain, myeloma, sarcoma and renal cancer. In 5 of the 20 cancer tissue sections, CACNA1D was downregulated and presented in the top 10% of downregulated genes (Fig. 2). In addition, CACNA1D levels also markedly declined in squamous cell lung carcinoma and lung adenocarcinoma, with fold changes ranging between-2.443 and -4.801, P-values ranging between $2.15x10^{-24}$ and $7.16x10^{-31}$ and gene rankings all in the top 1% relative to normal type-matched tissue. In brain tumors, consisting of glioblastoma, anaplastic astrocytoma and diffuse astrocytoma, CACNA1D was substantially downregulated, with fold changes between 2.814 and -2.850, P-values between 0.001 and $3.23x10^{-16}$ and gene rankings in the top 6-9%. To the best of our knowledge, these findings regarding the low expression of CACNA1D in myeloma, sarcoma and renal tumors (Table I) are novel findings. In our previous study (43), CACNA1D specifically had low expression in myeloma, sarcoma and renal tumors and was highly expressed in prostate, breast, colorectal, bladder, gastric, uterine and esophageal tumors. In addition, CACNA1D was up- and downregulated in brain and lung cancer. CACNA1D was upregulated in lung carcinoid tumors, but under-expressed in squamous cell lung carcinoma and lung adenocarcinoma. Based on the current data, CACNA1D may be a useful marker for potential tumor suppressor genes in cancer development; however, its mechanism of action in cancer requires additional investigation in prospective studies.

CACNA1F was reported to be involved in human physiology and photoreceptors, although its role in cancer biology is generally unknown. In the present analysis, CACNA1F was under-expressed in anaplastic large cell lymphoma with a fold change of -2.044, P-value of $1.14x10^{-12}$, and gene ranking in the top 1% (Table I). In our previous study, CACNA1F was over-expressed in testicular teratoma relative to normal matched tissue samples (72). Therefore, CACNA1F had increased mRNA expression in testis cancer but decreased expression in lymphoma. These findings indicated that cell context-specific alterations in CACNA1F expression may play a critical role in cancer biology.

P/Q-type calcium channel family. The P/Q calcium channel is encoded by Cav2.1 (CACNA1A), located in Purkinje cells and cerebellar granule cells (73). CACNA1A is known to be involved in neurological disease (74); compared with normal tissue, CACNA1A is downregulated in brain, colorectal, gastric and breast cancers (Fig. 1). In 4 of the 20 cancer types studied, the expression of CACNA1A was low, with a gene ranking in the top 10% of downregulated genes (Fig. 1). Brain tumors, consisting of glioblastoma, anaplastic astrocytoma, astrocytoma, atypical teratoid/rhabdoid tumor, malignant glioma, primitive neuroectodermal tumor, classic medulloblastoma, desmoplastic medulloblastoma and oligodendroglioma, all showed significant downregulation (P<0.05) of CACNA1A in comparison to type-matched tissues. *In silico* analysis of brain datasets showed a -2.084-fold change in CACNA1A expression levels, with P-values ranging between $2.84x10^{-4}$ and $8.77x10^{-18}$ and a gene ranking in the top 1-5%. Colorectal cancers, including colorectal carcinoma and colon adenoma, all showed the most significant decreases in expression of CACNA1A relative to control samples, with-2.807- to -3.376-fold

downregulation, P-values between $3.39x10^{-5}$ and $5.25x10^{-6}$ and gene rankings in the top 8-10% (Table II). Compared with our previous studies (43), CACNA1A specifically had low expression in colorectal, esophageal and gastric cancers, but also had high expression in leukemia, and sarcoma, ovarian, uterine, lung and cervical tumors. In addition, it was also identified that CACNA1A was up- and downregulated in brain tumors. Overall, our bioinformatics analysis verified that CACNA1A is likely to be useful as a possible cancer therapeutic target for colorectal, esophagus, gastric and breast cancers.

N-type calcium channel family. The N-type calcium channel is encoded by an α_1 subunit termed CACNA1B (Cav2.2), which is localized in the brain and peripheral nervous system. In neuropathic pain, CACNA1B serves to preserve neuronal firing and neurotransmitter release (74). However, CACNA1B has not yet been associated with cancer. The bioinformatics data derived in the present study regarding brain and breast cancer showed that CACNA1B ranked in the top 1 and 5% of genes with low expression, respectively. Brain tumors, including glioblastoma, oligodendroglia, anaplastic astrocytoma, diffuse astrocytoma and glioblastoma all showed significant downregulation (P<0.05) of CACNA1B relative to control samples. *In silico* analyses of brain datasets showed under-expression of CACNA1A, ranging between -4.336 and -8.235-fold decreases in transcript expression, P-values between $1.05x10^{-5}$ and $3.47x10^{-19}$ and gene rankings in the top 1-5%. For CACNA1B expression in male breast carcinoma, the data also showed decreases, with a -2.067-fold change and a P-value of $3.41x10^{-5}$ relative to control tissue (Table III). Compared with our previous results (43), CACNA1B specifically had low expression in brain cancer, but also had high expression in prostate tumors. In addition, CACNA1B was also upregulated in intraductal cribriform breast adenocarcinoma, but downregulated in male breast carcinoma. Finally, the present data indicated that CACNA1B was moderately expressed in brain cancer and male breast cancer. Understanding the underlying mechanisms behind CACNA1B action in cancer progression may assist in determining prospective therapeutic targets for the treatment of brain cancer and male breast cancer.

T-type calcium channel family. The T-type calcium channel consists of three subtypes [Cav3.1 (CACNA1G), Cav3.2 (CACNA1H) and Cav3.3 (CACNA1I)], which are located in neurons, pacemaker cells and osteocytes. The functions of the T-type calcium channel remain generally unknown. CACNA1G exhibited low expression in numerous cancers, including ovarian serous adenocarcinoma and mucinous breast carcinoma, with a gene ranking in the top 3-5% of genes with low expression, fold changes between -6.893 and -2.209, and P-values between $2.42x10^{-5}$ and $4.27x10^{-10}$ (Table IV). Compared with our previous results (43), CACNA1G specifically had low expression in ovarian, renal, brain and bladder cancer, but also exhibited high expression in sarcoma, lung, uterine and prostate tumors. CACNA1G was also revealed to be upregulated in invasive lobular breast carcinoma but downregulated CACNA1G was observed in mucinous breast carcinoma and mantle cell lymphoma. In addition, CACNA1G was upregulated in rectosigmoid adenocarcinoma but downregulated in colorectal carcinoma.

Table III. N-type calcium channel expression in cancers.

Gene and cancer type	Patient number, n	P-value (cancer/normal)	t-test (cancer/normal)	Fold (cancer/normal)	Gene ranking, %	Database reference	(Refs.)
CACNA1B							
Brain							
Glioblastoma	180	3.47×10^{-19}	-12.113	-8.235	656 (in top 4)	Cancer Cell 2006/04/01	(70)
Oligodendroglioma	180	2.37×10^{-11}	-7.840	-4.336	947 (top 5)	Cancer Cell 2006/04/01	(70)
Anaplastic astrocytoma	180	1.37×10^{-9}	-7.611	-5.134	223 (top 2)	Cancer Cell 2006/04/01	(70)
Diffuse astrocytoma	180	1.40×10^{-6}	-6.720	-4.929	33 (top 1)	Cancer Cell 2006/04/01	(70)
Glioblastoma	84	1.05×10^{-5}	-19.805	-5.922	691 (top 4)	J Clin Oncol 2008/06/20	(71)
Breast							
Male breast carcinoma	593	3.41×10^{-5}	-4.423	-2.067	827 (top 5)	TCGA2011/09/02	

CACNA1H was downregulated in gastrointestinal stromal tumor, sarcoma and renal cancer (Fig. 1). CACNA1H presented in the top 1-3% of downregulated genes in glioblastoma and renal oncocytoma, respectively. Compared with normal tissue, the fold changes ranged between -3.370 and -2.201, and P-values ranged between 7.01×10^{-13} and 1.76×10^{-15}. CACNA1H also exhibited low expression in bladder cancers, including infiltrating bladder urothelial carcinoma (fold change, -6.64; $P < 2.53 \times 10^{-15}$) and superficial bladder cancer (fold change, -4.59; $P = 1.49 \times 10^{-7}$). Notably, compared with our previous research, CACNA1H was specifically overexpressed relative to normal tissue samples in renal cancer, sarcoma and gastrointestinal stromal tumors (75). However, CACNA1H also had specific low expression in brain, ovarian, bladder, lung and breast cancer (Table IV).

CACNA1I exhibited low expression in invasive breast carcinoma and breast carcinoma stroma, with a gene ranking in the top 5 and 10%, P-values of 1.75×10^{-4} and 7.55×10^{-16}, and fold changes between -4.405 and -2.184, respectively (Table IV). Notably, in our previous study, CACNA1I was overexpressed in invasive breast cancer, myxoid/round cell liposarcoma and esophageal adenocarcinoma relative to normal tissue samples (75). Compared with normal tissue, CACNA1I exhibited low expression in brain tumors, including brain glioblastoma, glioblastoma and anaplastic oligodendroglioma. Therefore, CACNA1I was proposed as a potential therapeutic target for these specific cancer subtypes.

R-type calcium channel family. Cav2.3 (CACNA1E) is the only subtype of R-type calcium channels and is localized in cerebellar granule and brain cells. Compared with normal tissue, CACNA1E showed fold changes between -3.061 and -2.027, P-values between 2.62×10^{-5} and $5-3.18 \times 10^{-6}$, and a gene ranking in the top 1-3% of downregulated genes in gastric and brain cancer, respectively (Table V). CACNA1E was overexpressed in esophageal and uterine cancer relative to normal tissue samples (72,75); however, CACNA1E also had low expression in prostate, leukemia, and lung and breast cancers. Therefore, CACNA1E was proposed as a potential therapeutic target for these specific cancer subtypes.

VGCCs in clinical application. The present data established that the expression of calcium channel family genes in normal brain tissue is substantial in comparison to their expression in brain tumor tissue. Previous studies showed that calcium channel antagonists involved in the signaling pathway of meningioma may affect growth factor-mediated meningioma proliferation (75,76). Another study used human astrocytoma U-373 MG and human neuroblastoma SK-N-MC cell lines as models to investigate the effects of calcium channel antagonists, including verapamil, nifedipine and diltiazem, on cancer cell lines (77). Cell growth inhibition occurred with interference from agonist-induced intracellular Ca^{2+} mobilization (78). T-type (Cav3.1) calcium channel α_1 subunit expression was changed in human glioma (76,79).

The effects of CACNA1A have been interpreted to represent a type of neurological disorder (80). Variations in the CACNA1A gene have been well documented to result in certain neurological diseases, including familial hemiplegic migraine type 1, sporadic hemiplegic type 1 and spinocerebellar ataxia type

Table IV. T-type calcium channel expression in cancers.

Gene and cancer type	Patient number, n	P-value (cancer/normal)	t-test (cancer/normal)	Fold (cancer/normal)	Gene ranking, %	Database reference	(Refs.)
CACNA1G							
Ovarian							
Ovarian serous adenocarcinoma	53	4.27×10^{-10}	-8.514	-6.893	685 (top 5)	Cancer Sci 2009/08/01	(99)
Colorectal							
Colorectal carcinoma	82	6.45×10^{-7}	-6.447	-3.266	1,537 (top 8)	Clin Exp Metastasis 2010/02/01	(111)
Brain							
Anaplastic astrocytoma	180	4.26×10^{-6}	-5.111	-1.976	1,771 (top 10)	Cancer Cell 2006/04/01	(70)
Breast							
Mucinous breast carcinoma	593	2.42×10^{-5}	-7.311	-2.209	438 (top 3)	TCGA 2011/09/02	(98)
Mantle cell lymphoma	336	7.74×10^{-4}	-3.839	-2.085	207 (top 3)	Nat Genet 2005/04/01	
Renal							
Renal oncocytoma	92	1.76×10^{-15}	-3.710	-2.201	362 (top 3)	Clin Cancer Res 2005/08/15	(94)
Chromophobe renal cell carcinoma		1.24×10^{-7}	-9.936	-2.096	490 (top 4)	Clin Cancer Res 2005/08/15	(94)
Bladder							
Infiltrating bladder							
Urothelial carcinoma	157	2.53×10^{-15}	-9.913	-6.640	380 (top 4)	J Clin Oncol 2006/02/10	(100)
Superficial bladder cancer	157	1.49×10^{-7}	-5.754	-4.595	1,158 (top 10)	J Clin Oncol 2006/02/10	(100)
CACNA1H							
Brain							
Glioblastoma	101	7.01×10^{-13}	-13.798	-3.370	129 (top 1)	Cancer Cell 2006/05/01	(115)
Ovarian							
Ovarian serous adenocarcinoma	53	1.44×10^{-8}	-7.924	-9.621	1,021 (top 7)	Cancer Sci 2009/08/01	(99)
Bladder							
Infiltrating bladder urothelial carcinoma	256	4.81×10^{-9}	-6.150	-2.679	356 (top 2)	J Clin Oncol 2010/06/01	(116)
Superficial bladder cancer	256	2.62×10^{-8}	-5.851	-2.292	1,295 (top 7)	J Clin Oncol 2010/06/01	(116)
Lung							
Lung adenocarcinoma	203	1.43×10^{-4}	-4.251	-2.152	609 (top 8)	Proc Natl Acad Sci USA 2001/11/20	(117)
Squamous cell lung carcinoma	203	3.80×10^{-4}	-3.779	-4.318	423 (top 5)	Proc Natl Acad Sci USA 2001/11/20	(117)

Table IV. Continued.

Gene and cancer type	Patient number, n	P-value (cancer/normal)	t-test (cancer/normal)	Fold (cancer/normal)	Gene ranking, %	Database reference	(Refs.)
Breast							
Invasive breast carcinoma stroma	59	7.55×10^{-16}	-12.196	-2.184	1,871 (top 10)	Nat Med 2008/05/01	(18)
Invasive breast carcinoma	158	1.75×10^{-4}	-11.625	-4.405	758 (top 5)	Breast Cancer Res Treat 2011/03/04	(118)
Brain							
Brain glioblastoma	557	4.91×10^{-9}	-19.105	-8.779	946 (top 8)	TCGA 2013/06/03	
CACNA1I							
Brain							
Glioblastoma	557	3.13×10^{-5}	-8.788	-7.373	185 (top 2)	TCGA 2013/06/03	
Anaplastic oligodendroglioma	54	0.001	-5.915	-2.371	620 (top 5)	Cancer Res2005/10/01	(119)

6 (81-84). However, CACNA1A was revealed to be frequently expressed in adenocarcinomas and involved in cell proliferation and differentiation (85,86). CACNA1A under-expression in atypical teratoid/rhabdoid tumors (AT/RT), malignant glioma and primitive neuroectodermal tumors had P-values of 9.05×10^{-5}, 2.07×10^{-4} and 4.64×10^{-4}, and gene rankings in the top 1, 2 and 1%, respectively. These expression levels were listed in the top 1 percentile. AT/RT is a rare malignancy frequently identified in childhood. AT/RT may develop in the CNS, including the spinal cord (87).

CACNA1G was also identified as a novel tumor suppressor in lung cancer (86). CACNA1G has a significantly different expression in squamous cell carcinoma and adenocarcinomas (86). Furthermore, it plays a role in cell proliferation and differentiation (88). CACNA1G was inactivated in colorectal cancers, colorectal adenomas, gastric cancers and acute myelogenous leukemia (42). CACNA1C and CACNA1D are also involved in cancers and other types of disease. CACNA1D was under-expressed in VCaP-siERG cells (89). It was also proposed to be a biomarker in patients with prostate cancer (90). In addition, CACNA1C was under-expressed in hypoplastic left heart syndrome (91,92).

Cancer cell lines are widely accepted as *in vitro* models used to study tumorigenic molecular processes. They have been used to perform useful molecular and functional analyses to understand the role of signaling pathways in tumor initiation and progression and also to identify potential therapeutic targets (93). For this purpose, international cancer projects, including the CCLE, have currently created a wide collection of human cancer cell lines from different tumor types. To identify the expression of CACNA1S in various cell lines, genomic data from 967 cancer cell lines in the CCLE collection was analyzed. These data showed consistency with Fig. 2. CACNA1S was downregulated in kidney cell lines but exhibited high expression in brain and leukemia cell lines (Fig. 3). CACNA1C was downregulated in prostate and pancreatic cell lines but exhibited high expression in breast, skin and lymphoma cell lines. CACNA1D was downregulated in brain, CNS and renal cell lines but had high expression in breast, gastric and lung cell lines. CACNA1A was downregulated in brain cell lines but exhibited high expression in leukemia, ovarian and uterine cell lines. CACNA1B was downregulated in brain and breast cell lines, but exhibited high expression in lung cell lines. CACNA1G was downregulated in bladder and renal cell lines, but exhibited high expression in breast cell lines. CACNA1H was downregulated in bladder and brain cell lines, but had high expression in gastric and renal cell lines. CACNA1I was downregulated in brain cell lines, but exhibited high expression in breast cell lines. The CCLE data were used to confirm the differences in VGCC gene expression in cancer tissue (Fig. 3). These data may provide unprecedented potential for discovering novel biomarkers of cancer in the VGCC gene family.

Tumor suppressor genes show low or under-expression in tumor tissue compared with normal tissue. Tumor suppressor gene changes result in the onset and progression of cancer. Determining the expression of these genes may therefore clarify the underlying mechanisms of oncogenesis. However, the tumor suppressor genes that are essential in numerous types of cancer remain poorly understood (45). In the present

Table V. R-type calcium channel expression in cancers.

Gene and cancer type	Patient number, n	P-value (cancer/normal)	t-test (cancer/normal)	Fold (cancer/normal)	Gene ranking, %	Database reference	(Refs.)
CACNA1E							
Brain							
Glioblastoma	180	5.71×10^{-14}	-9.108	-7.000	1,580 (top 9)	Cancer Cell 2006/04/01	(70)
Anaplastic astrocytoma	180	2.25×10^{-6}	-5.372	-4.289	1,610 (top 9)	Cancer Cell 2006/04/01	(70)
Glioblastoma	84	3.18×10^{-6}	-9.375	-2.027	566 (top 3)	J Clin Oncol 2008/06/20	(71)
Gastric							
Gastric mixed adenocarcinoma	69	0.001	-5.207	-2.241	661 (top 4)	Eur J Cancer 2009/02/01	(113)
Gastric cancer	27	2.62×10^{-5}	-5.134	-3.061	26 (top 1)	Med Oncol 2010/12/04	(120)
Prostate							
Prostate carcinoma epithelia	101	7.00×10^{-6}	-4.835	-2.496	91 (top 1)	Nat Genet 2007/01/01	(101)
Prostatic intraepithelial neoplasia epithelia	101	3.10×10^{-4}	-3.873	-2.331	368 (top 4)	Nat Genet 2007/01/01	(101)
Leukemia							
Chronic lymphocytic leukemia	111	4.10×10^{-5}	-5.849	-2.097	402 (top 5)	J Clin Oncol 2004/10/01	(121)
Lung							
Small cell lung carcinoma	203	6.11×10^{-5}	-4.747	-4.842	554 (top 7)	Proc Natl Acad Sci USA 2001/11/20	(117)
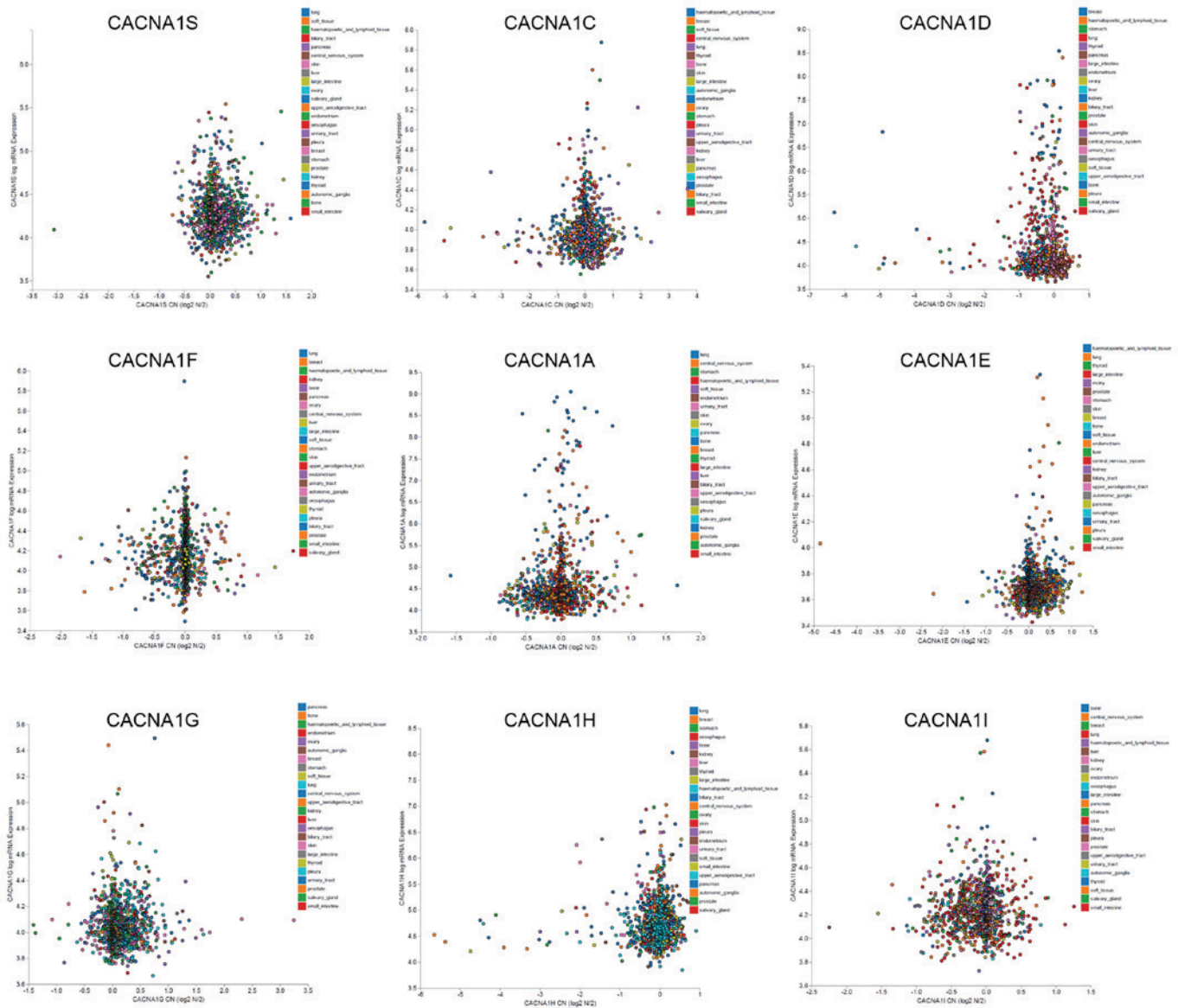
Figure 3. Plots showing voltage-gated calcium channel family gene mRNA expression z-scores vs. copy number value from 967 cell lines that were obtained through the Cancer Cell Line Encyclopedia. Each dot represents one cell line.

analysis, Oncomine was used to perform a meta-analysis of a clinical human microarray database. VGCCs were confirmed to play roles as tumor suppressor genes in specific cancers, including cancer tissue and cancer cell lines. Overall, VGCC family genes were under-expressed in numerous types of cancers, including brain, breast, kidney and lung cancers. Notably, the majority of VGCC family members (CACNA1C, CACNA1D, CACNA1A, CACNA1B, CACNA1E, CACNA1H and CACNA1I) were downregulated in brain tumors, with an mRNA expression level in the top 1-9% of the gene ranking. A total of 5 of the VGCC family members (CACNA1A, CACNA1B, CACNA1E, CACNA1G and CACNA1I) showed under-expression in breast cancer, with gene rankings in the top 1-10% of the expressed genes relative to normal tissues. In kidney cancer and lung cancer, CACNA1S, CACNA1C, CACNA1D, CACNA1A and CACNA1H exhibited low expression, with gene rankings in the top 1-8% of expressed genes. Collectively, the involvement of calcium channels in

cancer development and progression remains largely obscure, although numerous studies have investigated this topic. The present findings may help clarify the role of VGCCs as tumor suppressors in cancer development and may contribute to the development of new cancer treatment approaches for specific types and subtypes of cancer.

Acknowledgements

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