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No transcriptional evidence for active Na_v channels in two classes of cancer cell

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ABSTRACT

Voltage-gated sodium channel (Na_v) expression in non-excitable cells has raised questions regarding their non-canonical roles. Interestingly, a growing body of evidence also points towards the prevalence of aberrant Na_v expression in malignant tumors, potentially opening a new therapeutic window. In this study, the transcriptional consequences of channel inhibition were investigated in non-small cell lung carcinoma H460 and neuroblastoma SH-SYSY cell lines, that both express $Na_v1.7$. Channel activity was blocked by the application of both selective, ProTx-II, and non-selective, tetrodotoxin, inhibitors. Global gene expression profiling did not point to any statistically significant inhibition-associated perturbation of the transcriptome. A small subset of genes that showed relatively consistent changes across multiple treatments were further assayed in the context of a multiplex bead expression array which failed to recapitulate the changes seen in the global array. We conclude that there is no robust transcriptional signature associated with the inhibition of two sodium channel expressing cancer cell lines and consequently sodium channel inhibition will not lend itself to therapeutic approaches such as transcription-based drug repurposing.

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Introduction

Voltage-gated sodium (Na_v) channels were originally characterized as prominent players in signal conduction in excitable cells, such as neurons and myocytes, principally through the regulation of cellular ion balance [1,2]. Subsequent findings led to speculation that Na_v channels may also be involved in a wider repertoire of cellular processes [3]. One notable observation was that Na_v channels are not exclusively present in excitable cells and appear to take on roles that are not necessarily related to action potential generation and cellular excitability [4]. For example, functional expression of the channels has been observed in cancer cells, with recordings of voltage-gated sodium currents in human leukemia [5] and small-cell lung cancer cells [6]. Over the years, innumerable studies from independent research groups have added to a rapidly expanding body of evidence for the functional expression of Na_v channels in non-excitable cells of wide-ranging backgrounds including, red blood cells [7], macrophages [8], oligodendrocytes [9], dendritic cells [10], and fibroblasts [11]. The expression of Na_v channels in these cells is dynamic, and may alter depending on the developmental, physiological, and pathological state [12,13]. This dynamic expression profile has been linked to distinct biological states of the cell serving to implement diverse biological functions, e.g. motility, endosomal acidification, and phagocytosis [14,15].

The functional relevance of Na_v channels in a non-excitable context has been most intensely investigated in cancerous cells. This focus could certainly be explained in part by the fact that Na_v channels are anomalously expressed in a wide range of tumors, including, lymphoma, breast cancer, melanoma, colon, and ovarian cancer [16]. In some cases, the channels are not present in the corresponding healthy tissues, e.g., breast cancer [17]. On the other hand, in certain cancers, such as glioma and ovarian cancer, Na_v channels are expressed in the healthy counterparts [18,19]. In the latter case, the degree of channel overexpression is tied to the aggressiveness of cancer metastasis [20]. The mechanisms by which Na_v channels exert their metastasis-related effects are only

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beginning to be unraveled and thus far results have largely been obtained from breast and prostate cancer studies [21]. Sodium ions are clearly an important factor, still how much of the observed response is due to the fluctuation in Na^+ concentration, membrane potential, or transporter-assisted ion exchange, has yet to be fully characterized [22]. One school of thought believes the interaction between Na_v channels and partner proteins to be critical, based on accumulating evidence that various scaffolding proteins partner with the channels to form multiprotein complexes [23,24].

Na_v channels exist in several isoforms which are distributed in distinct cell types and locations, e.g., $Na_v1.1$ is expressed in both the CNS and PNS to $Na_v1.9$ predominantly in the PNS [25]. Mutations in $Na_v1.7$ are associated with painful channelopathies and congenital insensitivity to pain [26,27]; it comes as no surprise that this channel has been a prime target for pain management [28]. Interestingly, parallel work has revealed $Na_v1.7$ upregulation in a number of tumors, for example, breast [17], prostate [29], gastric [30], and non-small cell lung cancer [31]. This overexpression is reportedly associated with tumor-related transcriptional changes and is linked to the aggressive phenotypes exhibited by the metastatic cells [14,30,32]. It follows that suppression of $Na_v1.7$ expression or activity might improve cancer prognosis. In fact, there is speculation that the use of analgesics that engage Na_v channels for cancer surgery may have implications in lessening tumor reoccurrence [33].

In the present work, we have sought to elucidate the functional consequence of sodium channel expression in the cancer context by following changes in gene expression upon channel inhibition. The possibility that there are transcriptional changes associated with channel inhibition could potentially open up new avenue for therapeutic screening and possible drug repurposing [34–38] based on the wealth of available drug-associated transcriptional data [39]. $Na_v1.7$ has been shown to be the prominent Na_v subtype in the H460 lung cancer and SH-SY5Y neuroblastoma cell lines [31,40] and we decided to investigate channel activity in these two cell lines with a specific $Na_v1.7$ antagonist and a more general inhibitor. Specifically, the pufferfish derived neurotoxin tetrodotoxin (TTX) has been shown to block a subset of Na_v channels (TTX sensitive $Na_v1.1$–1.4 and $Na_v1.6$–1.7; Pinto, Derkach [41]), and the tarantula derived neurotoxin ProTx-II exhibits $Na_v1.7$ selectivity [42].

Methods

Pharmacological treatment and microarray

SH-SY5Y neuroblastoma cell line was kindly provided by Dr Paul Francis' group (Wolfson CARD, KCL UK), and maintained in DMEM-high glucose medium at 37°C, 5% CO_2. NCI-H460 non-small cell lung cancer cell line was purchased from ATCC USA and maintained in RPMI 1640-GlutaMAX medium at 37°C, 5% CO_2. Cells were passaged 2–3 times a week. Complete growth media for both cell lines were supplemented with 10% FBS (PAA Laboratories, UK), 100 U/mL penicillin, and 100 µg/mL streptomycin. All culture media and supplements were purchased from Sigma-Aldrich, UK, unless otherwise stated.

For the experiments, SH-SY5Y and H460 cells were seeded at 100,000 cells per well in a 12-well sterile cell culture plate and incubated for 48 h to reach approximately 80% confluence. Cells were then treated for 6 h with 1 µM TTX (Sigma-Aldrich), 1 or 10 nM ProTx-II (SmarTox); as the IC_{50} of ProTx-II for $Na_v1.7$ is approximately 1 nM [43], we decided to work with concentrations in the range 1 to 10 nM. Each treatment was replicated in four culture wells. Following this, culture media were removed, and the cells were lysed in Absolutely RNA Miniprep Kit lysis buffer and β-mercaptoethanol (Agilent Technologies, UK). RNA was then extracted, and quality assessed (RNA Integrity Number ≥8). RNA expression levels were measured on Affymetrix Human Genome U133 plus 2.0 (GPL570) chip following the supplier's recommended procedure. Resulting expression data were pre-processed with Affymetrix MAS5.0.

Multiplex assay

A custom-made set of paramagnetic microbeads comprising detection probes for 50 genes (44

chosen based on preliminary analysis and 6 housekeepers) was supplied by Luminex (Thermo Fisher Scientific). Assay components were stored at 2–8 °C or −20 °C according to storage specification.

For one well on a 96-well plate, working bead solution was made up by mixing 9.2 µL nuclease-free water, 6.6 µL lysis buffer, 1 µL blocking reagent, 0.2 µL proteinase K, 0.5 µL capture beads, and 2.5 µL probe set, totaling 20 µL. All reagents were supplied as part of the Plex Assay Kit (QuantiGene, UK). Eighty microliters of cell lysate and 20 µL working bead solution were mixed and added to each well. Three blanks and a positive control were included in every run. The assay plate was incubated for 18 h at 54°C, 600 rpm. After overnight hybridization, the plate was secured on a magnetic hand-held platform, and the assay wells were rinsed 3X with 100 µ wash buffer. One hundred microliters of pre-amplifier solution was added and incubated for 1 h at 50°C, 600 rpm. The incubation-wash step was repeated with an amplifier, and label probe solution. After 3X washes for label probe solution, 100 µL SAPE working reagent (3 µL SAPE per 1 mL SAPE diluent) was added to each assay well and incubated for 30 min at RT, 600 rpm. The wells were washed 3X with SAPE wash buffer. Lastly, 130 µL of SAPE wash buffer was added and mixed well by agitating at 800 rpm for 3 min. The plate was read immediately on the MAGPIX platform with xPONENT software (Luminex, USA).

Validation of cell type

The NCBI GEO hosts 145,000 samples on this platform, making it the most popular array chip. The relative expression levels of probes were collected for the GEO data and the given cell types. The ranks were scaled to lie between zero for the highest expression probe to unity for the lowest. The relative rank of each probe was defined as $\frac{r_0-r}{r_0}$ for $r < r_0$ and $\frac{r_0-r}{1-r_0}$ for $r > r_0$, where r and r_0 are the average probe ranks over the given cell type samples and the set of samples deposited on GEO, respectively. Probes were then mapped to genes and in the case of degeneracy, the probe with the largest relative rank mapping to the gene. The gene rank profile was taken to be related to the relative gene expression characterizing the cell type.

Microarray transcription profiles

Transcription profiles for sodium channel inhibition were based on within plate treatment versus control gene expression levels, where the scaled fold was defined as $2\frac{\langle t\rangle-\langle c\rangle}{\langle t\rangle+\langle c\rangle}$, were $\langle t\rangle$ and $\langle c\rangle$ are the average treatment and control levels, respectively. In the case of the cell type profiles, the averages were taken over the two cell types. Only folds over 20% passing a t-test threshold of 0.05 were considered. Heat maps for individual gene and sample pairs were generated based on the gene expression level relative to the distribution over all samples, $\frac{x-\langle x\rangle}{\sigma_x}$, where x is the expression level, the brackets indicate the average and the denominator is the standard deviation.

Bead array transcription profiles

The median level fluorescence of between 50 and 100 bead reads was taken to be the measure of the corresponding gene's RNA level. Gene expression levels in each well were normalized against the geometric mean of the total gene set. The normalization scheme was chosen to maximize the correlation of the cell type expression profile with that seen in the microarray data. Expression profiles were defined as for the microarray analysis, see above.

Results

Cell-specific nature of the microarray profiles

As a first step in establishing the phenotype of the model cell system, their transcriptional profile was queried against a database of publicly deposited gene expression profiles via SPIED [44,45], see Methods. The top 1000 genes in the H460 and SH-SY5Y or SHSY rank profiles are overwhelmingly up-regulated consisting of (977 up-regulated and 23 down-regulated) for H460 and (950 up-regulated and 50 down-regulated) for SHSY. These profiles served as queries in the SPIED search. It is perhaps worth pointing out

here that the level of gene expression unique to a given cell type will tend to be elevated relative to a background consisting of a variety of tissue types. An analogy would be in the context of division of labor one is characterized by what one does not by what one does not do. The top SPIED hits show a high correlation with appropriate cell types, validating the cell lineages, with the H460 query resulting in exclusively H460 profiles, Figure 1(a). The neuroblastoma query results are less exclusively that of the same cell type, but nonetheless picking out 12 profiles of related cell phenotype in the top 51, Figure 1(b).

Expression level of sodium channel types across cell types is shown in Figure 1(c). The lung cancer cells H460 only express SCN3A and SCN9A, whereas the neuroblastoma cells SHSY express SCN1A, SCN2A, SCN3A, and SCN9A.

Gene expression is not affected by channel inhibition

To investigate whether the modulation of channel activity has a transcriptional consequence, the extent of gene expression change was determined with samples segregated according to treatment type and compared to a random assignment of sample to treatment type. The extent to which expression is driven by the given treatment is then measurable by a Monte Carlo simulation, counting the number of times a random assignment has a larger perturbed gene set [46]. As can be seen from Figure 2(a,b), neither of the sodium channel inhibitors TTX or PTX pass a statistical threshold of treatment dependent gene perturbation. Individual profiles generated from samples from the various plate series were also compared for consistency in regulation. As can be seen from Figure 2(c) there appears to be little in the way of robustness in the conservation of regulated gene sets, with the numbers going in the same direction alternately surpassing and coming short of those going in opposite directions. It can be concluded that there is no robuts transcriptional signature associated with sodium channel inhibition of the the two cancer cell types, with the caveat that the transcriptional effects would be more pronounced with higher inhibitor concentrations. However, as with all pharmacological studies, one must be wary of non-

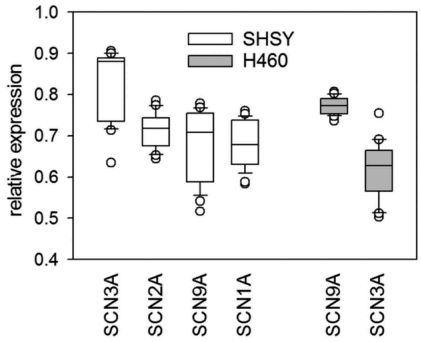
a

RANK	correl	ln(prob)	description	SERIES	SAMPLES
1	0.95	3.23E-132	H460	GSE51739	GSM1251844
2	0.98	1.80E-127	H460	GSE10843	GSM274737
3	0.97	4.68E-103	H460	GSE57083	GSM1374746
4	0.97	8.01E-94	H460	GSE8332	GSM206493
6	0.96	9.88E-92	H460	GSE34211	GSM844652
7	0.96	9.11E-88	H460	GSE15126	GSM378005
9	0.85	2.71E-87	H460	GSE4824	GSM108853
10	0.8	7.01E-89	H460	GSE32474	GSM803771
12	0.93	4.96E-69	H460	GSE14315	GSM357883
13	0.71	5.16E-89	H460	GSE36176	GSM882375
15	0.94	4.26E-65	H460	GSE41445	GSM1017497
16	0.92	5.68E-66	H460	GSE5816	GSM134927

b

RANK	correl	ln(prob)	description	SERIES	SAMPLES
2	0.9	7.92E-74	neuroblastoma	GSE68950	GSM1688009
3	0.91	4.90E-73	SHSY5Y	GSE18676	GSM463943
7	0.71	1.12E-62	neuroblastoma	GSE39262	GSM959014
16	0.64	2.44E-56	glioblastoma	GSE1993	GSM35744
23	0.76	5.94E-43	SHSY5Y	GSE7403	GSM178593
25	0.65	2.93E-50	glioblastoma	GSE13041	GSM326886
27	0.58	9.05E-54	brain	GSE14108	GSM354030
29	0.59	1.22E-52	neuroblastoma	GSE3506	GSM80033
32	0.55	9.70E-56	foetal cortex	GSE15209	GSM379866
34	0.73	4.22E-42	ependymoma	GSE21687	GSM541119
43	0.54	2.12E-51	glioblastoma	GSE13041	GSM326909
51	0.6	3.33E-43	neuroblastoma	GSE53046	GSM1281030

c



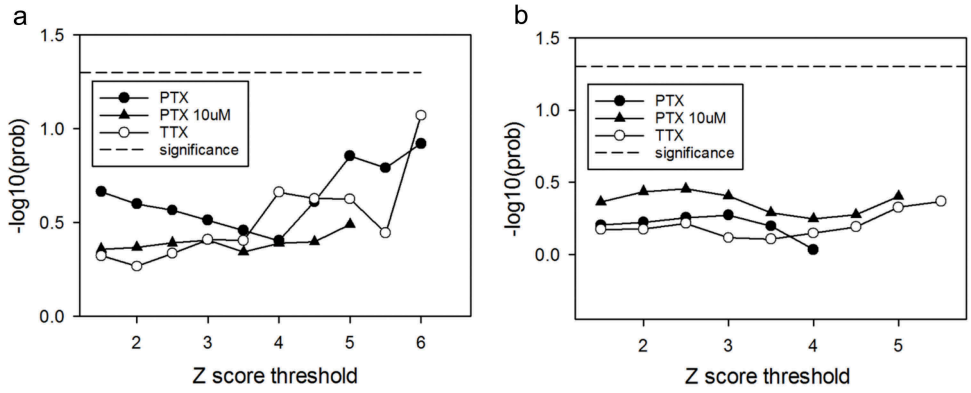
SHSY

GENE	probe ID	expression	RANK
SCN3A	210432_s_at	0.83	831
SCN2A	229057_at	0.71	2123
SCN9A	229199_at	0.68	2659
SCN1A	210383_at	0.68	2694

H460

GENE	probe ID	expression	RANK
SCN9A	206950_at	0.77	1199
SCN3A	210432_s_at	0.62	3439

Figure 1. The relative rank expression profile for the H460 (a) and SHSY (b) samples were queried against human samples in SPIED. The top hits are listed in the table, showing that they are enriched by samples from corresponding cell types. The correlation is more pronounced for the H460 cells as these are the subject of more available studies. The SHSY correlations pick out SHSY samples as well as samples from various brain cancer cells. The two cell lines express the sodium channels SCN3A and SCN9A (c). The neuroblastoma cells also express SCN1A and SCN2A.



c

	SHSY				H460				
	PTX 10nM	PTX 10nM	TTX 1µM	TTX 1µM	PTX 1nM	PTX 5nM	PTX 10nM	TTX 1µM	TTX 1µM
PTX 1nM	-	36,53	82,49	35,40	44,98	63,35	57,67	55,58	43,79
PTX 10nM		67,52	114,55	52,46	74,77	73,63	97,57	54,87	66,71
PTX 10nM			74,38	-	103,41	39,84	108,41	54,70	63,34
TTX 1µM				47,57	80,84	70,71	76,98	85,66	67,67
TTX 1µM					62,45	36,41	61,37	51,42	51,33
PTX 1nM						71,59	-	59,94	-
PTX 5nM							66,70	-	46,71
PTX 10uM								74,100	-
TTX 1µM									46,66

Figure 2. There is no significant transcriptional response upon inhibiting sodium channels in either the lung cancer or neuroblastoma cell lines. Monte Carlo significance test of association of gene expression with treatment type shows that neither the SHSY response (a) or H460 response (b) is significantly elevated beyond a random association of treatment and control groups. The inhibitor expression profiles generated from independent plate replicates show little consistency, see (c). The entries are colored according to the sign of the correlation, green for positive and orange for negative.

specificity emerging here, see Park, Carlin [43] and Pinto, Derkach [41]

Sodium channel gene set

The overall gene expression profiles point to there being little or no effect of channel modulation in either cell type examined. Nonetheless, there may be a restricted gene set that is consistently perturbed by channel modulation. To this end, the separate profiles defined according to cell type, treatment type, and concentration were generated and compared, see Figure 3(a). There is a small population of genes that are consistently perturbed across treatments and it was reasoned that these could facilitate a biomarker for channel modulation. As a positive control for subsequent verification, a gene set was designed based on delimiting the two cell types, see Figure 3(b).

Bead assay

Comparison of cell type-specific expression is in good agreement with the array data, Figure 4 explaining this. The bead assay expression data are given in Figure 5. It is clear that there is no correlation between predicted expression direction change and that observed across 15 independent sodium channel inhibition treatments.

Conclusions

This study sought to investigate the activity of sodium channels in the context of cancer cells through transcriptional profiling. We first

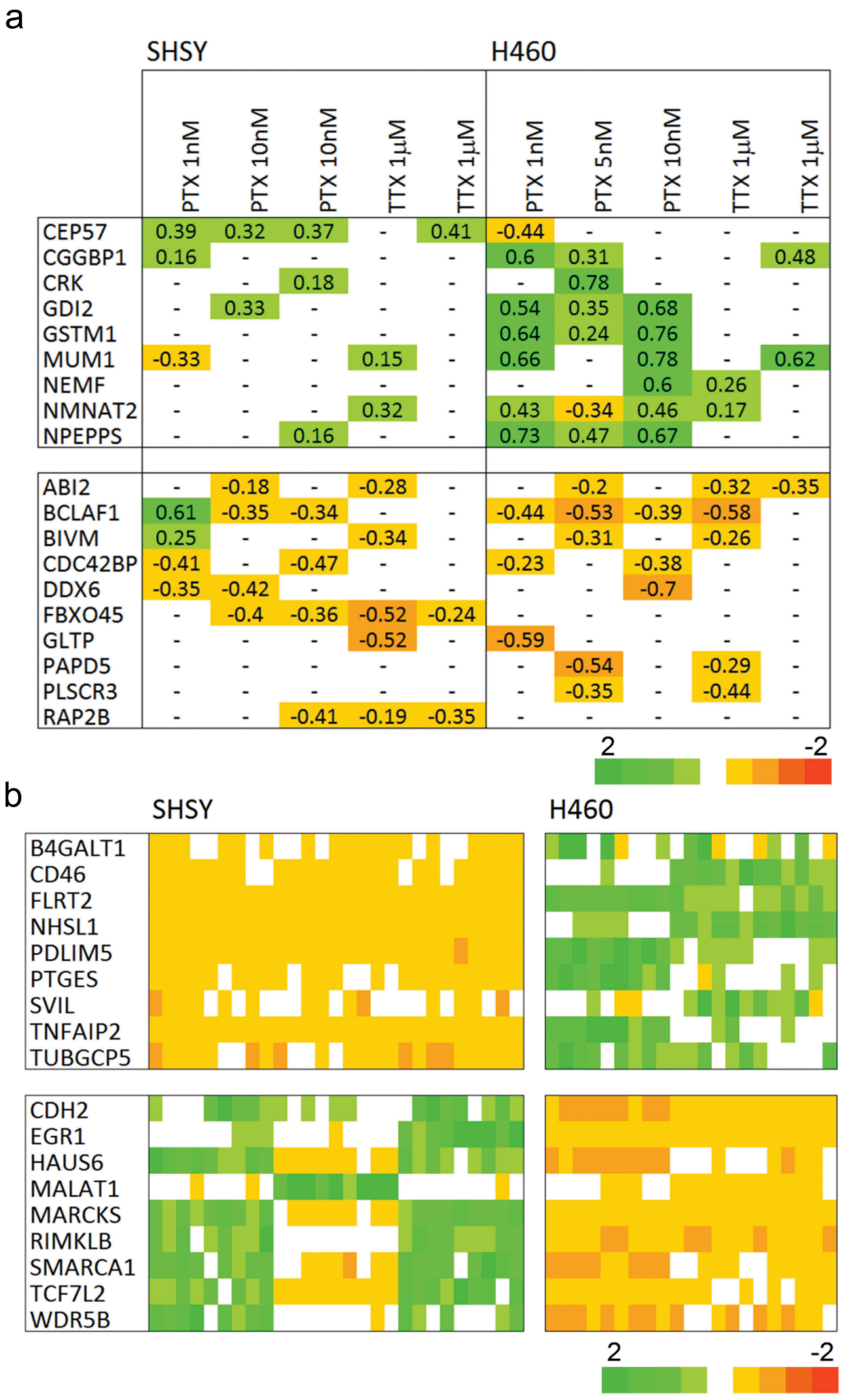


Figure 3. The gene sets for validation. The gene set based on consistently regulated genes by the PTX and TTX inhibitors are shown in (a), the cells are colored according to the scaled fold value, see Methods. Cell type is strongly encoded in relative gene expression (b) and can serve as a positive control for the multiplex array experiments. The cells are colored according to the Z-score of the response relative to the average and standard deviation over the full sample set, $\frac{x-\langle x\rangle}{\sigma_x}$, where x is the expression level, the brackets indicate the average and the denominator is the standard deviation.

Figure 4. The cell type tracker set effectively segregates the lung cancer and neuroblastoma cells. The cells are colored according to the Z-score of the response relative to the average and standard deviation over the full sample set, $\frac{x-\langle x\rangle}{\sigma_x}$, where x is the expression level, the brackets indicate the average and the denominator is the standard deviation.

showed that the H460 and SH-SY5Y cell phenotype could be effectively defined through relative gene expression and further showed that a subset of sodium channels is expressed in the two cell types. Having established sodium channel expression, we then determined whether pharmacological intervention would elicit a transcriptional response by applying inhibitor toxins that block Na_v channels either non-selectively (TTX) or with Nav1.7 specificity (ProTx-II). Our initial microarray results suggested that these inhibitors do not induce consistent alterations in overall gene expression in either of the two cell lines as indicated by Monte Carlo significance test and inter-profile comparison. Nonetheless, we reasoned that there might still be a restricted gene set that responds to channel modulation and selected a subset of genes from the microarray data for further validation in a multiplex platform. Two distinct multiplex biomarker gene sets were defined to, first of all, validate the platform through cell-specific marker expression and subsequently to test the validity of inhibitor-associated changes observed in the microarray data. The multiplex platform successfully delimited the cell type based on the expression of cell type marker genes but failed to reproduce the putative inhibitor-associated changes. We, therefore, conclude that in the context of gene expression there is no robust sodium channel activity in the two cancer cell lines under normal culture conditions. Consequently, assuming channel inhibition is a potential therapeutic target in cancer our results indicate that transcription-based approaches are unlikely to succeed. However, there is a possibility that the sodium channel activity is transcriptionally silent or that expression changes are not substantial enough for the two independent platforms to detect. For example, by altering membrane potential the channels might cause localized calcium influx and kinase activation in a manner that might impact on migration and/or proliferation in the absence of substantial transcriptional changes. Further, it must be borne in mind that our results are based on cells grown under normal culture conditions and there remains the

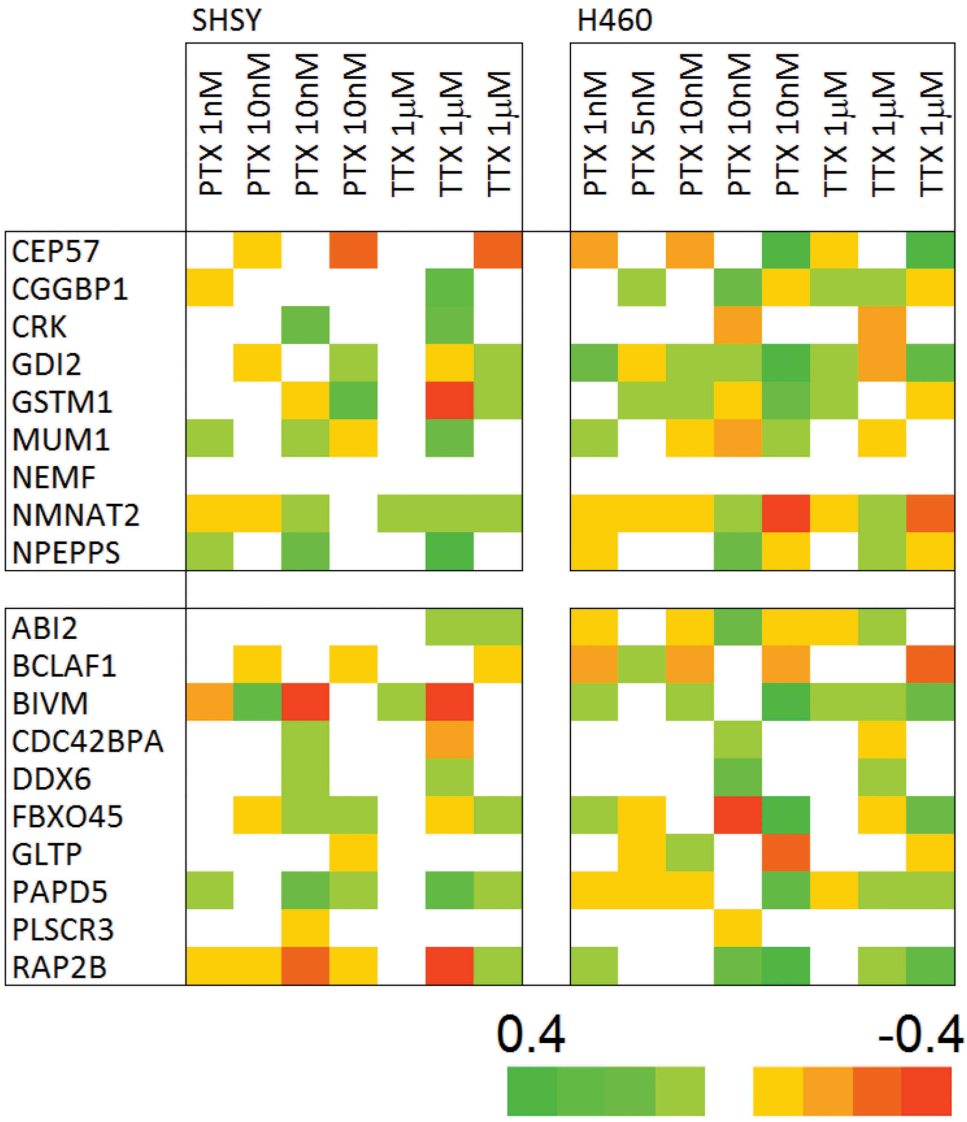


Figure 5. Bead array channel modulator tracker shows no association with sodium channel inhibition. The association of gene expression change with sodium channel inhibition seen in the array experiments, Figure 3(a) is not recapitulated in the bead experiment. The cells are colored according to the scaled fold value, see Methods.

possibility that the apparently silent channels might be activated by as yet to be identified agents and/or conditions.

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

[1] Hodgkin AL, Huxley AF. A quantitative description of membrane current and its application to conduction and excitation in nerve. J Physiol. 1952;117(4):500–544.
[2] Hille B. Ionic channels in excitable membranes. Current problems and biophysical approaches. Biophys J. 1978;22(2):283–294.
[3] Savio Galimberti E, Gollob M, Darbar D. Voltage-gated sodium channels: biophysics, pharmacology, and related channelopathies. Front Pharmacol. 2012;31.
[4] Black JA, Waxman SG. Noncanonical roles of voltage-gated sodium channels. Neuron. 2013;80(2):280–291.
[5] Yamashita N, Hamada H, Tsuruo T, et al. Enhancement of voltage-gated Na^+ channel current

associated with multidrug resistance in human Leukemia cells. Cancer Res. 1987;47(14):3736–3741.
[6] Pancrazio JJ, Viglione MP, Tabbara IA, et al. Voltage-dependent ion channels in small-cell lung cancer cells. Cancer Res. 1989;49(21):5901–5906.
[7] Hoffman JF, Dodson A, Wickrema A, et al. Tetrodotoxin-sensitive Na+ channels and muscarinic and purinergic receptors identified in human erythroid progenitor cells and red blood cell ghosts. Proc Natl Acad Sci U S A. 2004;101(33):12370–12374.
[8] Craner MJ, Damarjian TG, Liu S, et al. Sodium channels contribute to microglia/macrophage activation and function in EAE and MS. Glia. 2005;49(2):220–229.
[9] Tong X-P, Li X-Y, Zhou B, et al. Ca(2+) signaling evoked by activation of Na(+) channels and Na(+)/Ca(2+) exchangers is required for GABA-induced NG2 cell migration. J Cell Biol. 2009;186(1):113–128.
[10] Zsiros E, Kis-Toth K, Hajdu P, et al. Developmental switch of the expression of ion channels in human dendritic cells. J Immunol. 2009;183(7):4483–4492.
[11] Chatelier A, Mercier A, Tremblier B, et al. A distinct de novo expression of Nav1.5 sodium channels in human atrial fibroblasts differentiated into myofibroblasts. J Physiol. 2012;590(17):4307–4319.
[12] Black JA, Newcombe J, Waxman SG. Astrocytes within multiple sclerosis lesions upregulate sodium channel Nav1.5. Brain. 2010;133(Pt 3):835–846.
[13] Paez PM, Fulton D, Colwell CS, et al. Voltage-operated Ca(2+) and Na(+) channels in the oligodendrocyte lineage. J Neurosci Res. 2009;87(15):3259–3266.
[14] Brackenbury WJ, Djamgoz MBA, Isom LL. An emerging role for voltage-gated Na(+) channels in cellular migration: regulation of central nervous system development and potentiation of invasive cancers. Neuroscientist. 2008;14(6):571–583.
[15] Black JA, Newcombe J, Waxman SG. Nav1.5 sodium channels in macrophages in multiple sclerosis lesions. Mult Scler. 2013;19(5):532–542.
[16] Roger S, Potier M, Vandier C, et al. Voltage-gated sodium channels: new targets in cancer therapy? Curr Pharm Des. 2006;12(28):3681–3695.
[17] Fraser SP, Diss JKJ, Chioni A-M, et al. Voltage-gated sodium channel expression and potentiation of human breast cancer metastasis. Clin Cancer Res. 2005;11(15):5381–5389.
[18] Black JA, Liu S, Waxman SG. Sodium channel activity modulates multiple functions in microglia. Glia. 2009;57(10):1072–1081.
[19] Gao R, Shen Y, Cai J, et al. Expression of voltage-gated sodium channel alpha subunit in human ovarian cancer. Oncol Rep. 2010;23(5):1293–1299.
[20] Fiske JL, Fomin VP, Brown ML, et al. Voltage-sensitive ion channels and cancer. Cancer Metast Rev. 2006;25(3):493–500.
[21] Roger S, Gillet L, Le Guennec J-Y, et al. Voltage-gated sodium channels and cancer: is excitability their primary role? Front Pharmacol. 2015;6:152.
[22] Litan A, Langhans SA. Cancer as a channelopathy: ion channels and pumps in tumor development and progression. Front Cell Neurosci. 2015;9:86.
[23] Lee A, Fakler B, Kaczmarek LK, et al. More than a pore: ion channel signaling complexes. J Neurosci. 2014;34(46):15159–15169.
[24] Besson P, Driffort V, Bon É, et al. How do voltage-gated sodium channels enhance migration and invasiveness in cancer cells?. Biochim Biophys Acta. 2015;1848(10 Pt B):2493–2501.
[25] de Lera Ruiz M, Kraus RL. Voltage-gated sodium channels: structure, function, pharmacology, and clinical indications. J Med Chem. 2015;58(18):7093–7118.
[26] Yang Y, Wang Y, Li S, et al. Mutations in SCN9A, encoding a sodium channel alpha subunit, in patients with primary erythermalgia. J Med Genet. 2004;41(3):171–174.
[27] Cox JJ, Reimann F, Nicholas AK, et al. An SCN9A channelopathy causes congenital inability to experience pain. Nature. 2006;444(7121):894–898.
[28] King GF, Vetter I. No gain, No Pain: naV1.7 as an analgesic target. ACS Chem Neurosci. 2014;5(9):749–751.
[29] Diss JK, Stewart D, Pani F, et al. A potential novel marker for human prostate cancer: voltage-gated sodium channel expression in vivo. Prostate Cancer Prostatic Dis. 2005;8(3):266–273.
[30] Xia J, Huang N, Huang H, et al. Voltage-gated sodium channel Nav1.7 promotes gastric cancer progression through MACC1-mediated upregulation of NHE1. Int J Cancer. 2016;139(11):2553–2569.
[31] Roger S, Rollin J, Barascu A, et al. Voltage-gated sodium channels potentiate the invasive capacities of human non-small-cell lung cancer cell lines. Int J Biochem Cell Biol. 2007;39(4):774–786.
[32] Campbell TM, Main MJ, Fitzgerald EM. Functional expression of the voltage-gated Na(+)-channel Nav1.7 is necessary for EGF-mediated invasion in human non-small cell lung cancer cells. J Cell Sci. 2013;126(Pt 21):4939–4949.
[33] Fraser SP, Foo I, Djamgoz MB. Local anaesthetic use in cancer surgery and disease recurrence: role of voltage-gated sodium channels? Br J Anaesth. 2014;113(6):899–902.
[34] Lamb J, Crawford ED, Peck D, et al. The Connectivity Map: using gene-expression signatures to connect small molecules, genes, and disease. Science. 2006;313(5795):1929–1935.
[35] Wei G, Twomey D, Lamb J, et al. Gene expression-based chemical genomics identifies rapamycin as a modulator of MCL1 and glucocorticoid resistance. Cancer Cell. 2006;10(4):331–342.
[36] Zhang D, Ciciriello F, Anjos SM, et al. Ouabain mimics low temperature rescue of F508del-CFTR in cystic fibrosis epithelial cells. Front Pharmacol. 2012 Oct 4;3:176.

[37] Sirota M, Dudley JT, Kim J, et al. Discovery and preclinical validation of drug indications using compendia of public gene expression data. Sci Transl Med. 2011;3(96):96ra77.

[38] Dudley JT, Sirota M, Shenoy M, et al. Computational repositioning of the anticonvulsant topiramate for inflammatory bowel disease. Sci Transl Med. 2011;3(96):96ra76.

[39] Subramanian A, Narayan R, Corsello SM, et al. A next generation connectivity map: L1000 platform and the first 1,000,000 profiles. Cell. 2017;171(6): 1437–1452.e17.

[40] Vetter I, Mozar CA, Durek T, et al. Characterisation of Na(v) types endogenously expressed in human SH-SY5Y neuroblastoma cells. Biochem Pharmacol. 2012;83(11):1562–1571.

[41] Pinto V, Derkach VA, Safronov BV. Role of TTX-sensitive and TTX-resistant sodium channels in Aδ- and C-fiber conduction and synaptic transmission. J Neurophysiol. 2008;99(2):617–628.

[42] Schmalhofer WA, Calhoun J, Burrows R, et al. ProTx-II, a selective inhibitor of NaV1.7 sodium channels, blocks action potential propagation in nociceptors. Mol Pharmacol. 2008;74(5):1476–1484.

[43] Park JH, Carlin KP, Wu G, et al. Studies examining the relationship between the chemical structure of protoxin II and its activity on voltage gated sodium channels. J Med Chem. 2014;57(15):6623–6631.

[44] Williams G. A searchable cross-platform gene expression database reveals connections between drug treatments and disease. BMC Genomics. 2012;13:12.

[45] Williams G. SPIEDw: a searchable platform-independent expression database web tool. BMC Genomics. 2013;14:765.

[46] Golub TR, Slonim DK, Tamayo P, et al. Molecular classification of cancer: class discovery and class prediction by gene expression monitoring. Science. 1999;286(5439):531–537.