Acta Neuropsychiatrica

Acta Neuropsychiatrica 2012: 24: 16–25
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DOI: 10.1111/j.1601-5215.2011.00583.x

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ACTA NEUROPSYCHIATRICA

SOX11 target genes: implications for neurogenesis and neuropsychiatric illness

Sha L, Kitchen R, Porteous D, Blackwood D, Muir W, Pickard B. SOX11 target genes: implications for neurogenesis and neuropsychiatric illness.

Objective: Deficits in adult and embryonic neurogenesis have been linked with neurological and psychiatric disorders, so it is important to understand the molecular mechanisms underlying this process. SOX11 is a transcription factor known to play a critical role in the regulation of the neuronal and glial differentiation stage of neurogenesis, so we hypothesised that the identification of its target genes would reveal underlying biological processes relevant to disease.
Methods: SOX11 protein was over-expressed in HEK293 cells and transcriptional changes assessed by microarray analysis. Selected candidate genes were further tested for SOX11 activation in quantitative reverse transcriptase PCR studies of HEK293 cells and Western analysis of SH-SY5Y cells.
Results: Regulated genes included a previously established SOX11 target, known markers of neurogenesis, as well as several genes implicated in neuropsychiatric disorders. Immunofluorescence localised several of the genes within the proliferative subgranular zone of the hippocampus. We observed multiple histone and zinc finger genes regulated by SOX11, many of which were located in two clusters on chromosomes 6 and 19. The chromosome 6 cluster lies within a region of the genome showing the strongest genetic association with schizophrenia.
Conclusion: SOX11 appears to regulate a complex programme of chromatin remodelling and downstream gene expression changes to achieve a mature neuronal phenotype. SOX11 target genes are shown to be involved in neurodevelopmental processes important in health and, potentially, disease.

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Keywords: microarray analysis; nervous system disorders; transcription factor

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Significant outcomes

- Target genes of the important neurodevelopmental transcription factor, SOX11, have been identified through microarray analysis of cells over-expressing this gene.
- Many target genes participate in neuronal differentiation and neuropsychiatric disease processes.
- SOX11 regulates two clusters of zinc finger/histone proteins suggesting regional expression regulation.

Limitations

- Use of non-neuronal cell lines may prevent identification of the complete spectrum of SOX11 target genes.

https://doi.org/10.1111/j.1601-5215.2011.00583.x Published online by Cambridge University Press

Introduction

The SOX family of proteins are important transcription factors participating in the complex regulatory network that governs cellular proliferation and differentiation. The family is divided into subgroups on the basis of sequence homology and different functional roles (1). For example, SOX2 (SOXB1 group) promotes proliferative events in stem cells and is used in the reprogramming transcription factor cocktail that induces pluripotency in differentiated somatic cells (2–4). Members of the SOXB2 class are involved in the decision to exit proliferation and start the differentiation process (5).

SOX11 (Sry box-containing member 11), a member of the SOXC group, is mainly expressed in developing neurons, oligodendrocytes and astrocytes in human brain (6,7). Neurogenesis, in its full sense, involves the proliferation of adult neural stem cells or progenitors, the fate determination of these progenitor cells followed by survival, maturation and integration of the differentiating neurons into brain circuitry. It is in these later stages that the accumulated literature suggests SOX11 acts. For example, SOX11 regulates the establishment of pan-neuronal protein expression by inducing expression of neuronal markers in self-renewing precursor cells (8).

The 'neurodevelopmental' hypothesis of neuropsychiatric disorders such as schizophrenia, bipolar disorder and autism suggests that deficits in the proliferation, differentiation and connectivity of neurons during the formation of the brain might contribute towards increased risk of illness. Supporting a role for SOX11 in this context is a case report that recently described a 7-year-old patient with autism, moderate mental retardation, secondary microcephaly, agenesis of right optic nerve and dysmorphic features who carried a deletion of the *SOX11* gene (9). Additionally, we have identified *SOX11* as a negatively regulated target gene of the NPAS3 transcription factor (Sha et al., in press). Human genetics studies have implicated disruption (10) or variation (11) in the *NPAS3* gene as a risk factor for schizophrenia and bipolar disorder, respectively. The deficits in adult hippocampal neurogenesis present in a mouse *Npas3* knockout model (12) fit with the observations that reduced levels of adult neurogenesis are associated with schizophrenia, and possibly depression, and that greater levels are induced by drugs intended to treat these conditions (13,14).

Materials and methods

Gene cloning

The *SOX11* gene was cloned using the Invitrogen Gateway system, Paisley, UK. The human *SOX11* open reading frame was amplified from SH-SY5Y cell line complementary DNA (cDNA) using primers containing attB sites, cloned into the intermediate pDONR vector and then shuttled into the pDEST40 mammalian expression vector. Sequence verification of the clone open reading frame was carried out.

Cell culture and transient transfection

HEK293 cells were cultured in DMEM (Invitrogen, Carsbad, CA, USA) supplemented with 10% fetal bovine serum (Lab Tech Int., Ringmer, UK) at 37 °C in an atmosphere of 5% CO_2 in humidified air. The HEK293 cells were transiently transfected by pDEST40-SOX11 or control plasmid (pDEST40) using Lipofectamine™ 2000 transfection kit (Invitrogen) according to the manufacturer's instructions. After 4–6 h, the transfection medium was replaced with standard culture medium. Cells were collected at 24 h post-transfection. SH-SY5Y cells were also cultured and transiently transfected in the same manner for the western experiments.

Illumina microarray

Extraction of total RNA from transfected and control HEK293 cells was performed with the RNeasy Mini Kit (Qiagen, Valencia, CA, USA). Biotinylated cRNA was prepared using the Illumina TotalPrep RNA application kits (Ambion Inc., Austin, TX, USA) according to the manufacturer's direction starting with 100 ng total RNA. RNA and cRNA probe size ranges and purity were evaluated using Agilent 2100 bioanalyser (Agilent Technologies UK Ltd., Edinburgh, UK).

An Illumina Beadstation platform was used in conjunction with Sentrix® HumanRef-8 v2 chips (Illumina Inc., San Diego, CA, USA) capable of examining expression of over 24 500 gene transcripts. Hybridisation, washing and scanning were performed according to the Illumina BeadStation 500* manual (revision C) by experienced staff located within the Genetics Core of the Wellcome Trust Clinical Research Facility at the Western General Hospital, Edinburgh.

Analysis of microarray data

Expression differences between the cell lines were assessed using two Bioconductor (15) algorithms implemented in the statistical programming language, R. Genes differentially expressed between cell line samples were identified using *limma* and significance analysis of microarray (SAM) (16). The latter was used as part of the *BRB-Array Tools (3.70)* freeware developed by the Biometric

https://doi.org/10.1111/j.1601-5215.2011.00583.x Published online by Cambridge University Press

Research branch of the US National Cancer Institute (http://linus.nci.nih.gov/BRB-Arraytools.html). In both analyses, the Illumina probe profile expression data were log-transformed and normalised using quantile normalisation. For the analysis using the *limma* package, genes were defined as being differentially expressed after satisfying a minimum fold change of ±1.5 and a maximum, Benjamini-Hochberg adjusted, p-value of 0.01. For the SAM analysis, the differentially expressed genes were selected at a maximum predicted false discovery rate of 0.01. Regulated genes were further categorised by bioinformatics tools such as *Ingenuity Pathway Analysis* (Ingenuity Systems, Redwood City, CA, USA https://analysis.ingenuity.com/pa/login/applet.jsp) and *GeneCodis2* (http://genecodis.dacya.ucm.es/) for particular gene ontologies, biological processes and associated canonical pathways (17,18).

Microarray data have been submitted to *ArrayExpress* (http://www.ebi.ac.uk/arrayexpress/) with accession: E-TABM-1025.

Confirmation by QPCR

Quantitative reverse transcriptase PCR (QPCR) was used to validate microarray results using the same panel of HEK293 RNA and, separately, measure time-dependent changes in gene expression levels because of SOX11 over-expression at 0, 3, 6, 12, 24 and 48 h of transient transfection. cDNA was synthesised with reverse transcriptase (Roche, Welwyn Garden City, UK). QPCR was performed with SYBR green QPCR Master Mix (Invitrogen) and a Real-Time QPCR machine (BIO-RAD Laboratories Ltd., Hemel Hempstead, UK). Primers used in QPCR were designed using *Primer3 Software* and sequences are included in Table S2 (19). The housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) was selected as the endogenous references in this QPCR studies because it showed no SOX11-dependent expression change in the microarray data. For relative quantification of mRNA expression, geometric means were calculated using the comparative double delta method (20) with three replicates per time point. Student's t-test was used to determine significance at each time point and changes of $p < 0.05$ were considered significant.

Confirmation by Western

Samples prepared from SH-SY5Y cells were subjected to SDS-PAGE gel electrophoresis (NuPAGE® Novex® Tris-Acetate gels, Invitrogen, Paisley, UK). Protein was transferred to 0.2 μm Polyvinylidene Difluoride (PVDF) membrane (Invitrogen), blocked in 5% dried milk powder in 50 mM Tris–HCl, pH 7.5, 15 mM NaCl, 0.5% Tween-20 (TBST), after several wash and incubated in primary antibody solutions in 2% donkey serum, and incubated in these solutions for overnight at 4 °C. The following antibody solutions and dilutions were used: 1/1000 SOX11 rabbit anti-human, 1/1000 YWHAZ rabbit anti-human (loading control selected on the basis that its transcript showed no SOX11-dependent expression change by microarray), 1/800 SCG2 goat anti-human (Santa Cruz Biotechnology) and 1/800 TUBB3 rabbit anti-human (Abcam, Cambridge, UK). Blots were then washed three times in TBST, incubated for 1 h in anti-rabbit or anti-goat horse radish peroxidase-conjugated secondary antibody (1:1000 in TBST), after three wash and incubated in ECL plus Western blotting detection reagent for 1–5 min based on the manufacturer's instructions (GE Healthcare, Buckinghamshire, UK).

Immunofluorescence

Sections frozen mouse brain were cut at 10 μM using a Leica VT1000S vibratome (Leica Microsystems UK Ltd, Milton Keynes, UK). The sections were fixed in ice cold acetone for 5 min, air dried for 30 min and then washed in 0.1% Triton X-100 in phosphate-buffered saline (pH 7.4). The sections were first incubated for 1 h in 2% donkey serum. The sections were then incubated in primary antibody solution for overnight at 4 °C. The following antibody solutions were used: 1/500 Sox11 rabbit anti-mouse and 1/400 Gpc2 goat anti-mouse (Santa Cruz Biotechnology Inc., Heidelberg, Germany). Following several washes over the period of 1 h, the sections were incubated for 1 h with 1:400 donkey secondary antibodies against goat or rabbit IgG, conjugated to Alexa Fluor 594 for red fluorescence or FITC for green fluorescence (Invitrogen Life Technologies, Paisley, UK). Finally, after several washes, the sections were mounted with DAPI (Invitrogen). Images were captured using SmartCapture 2 version 3 software (Digital Scientific Ltd, Cambridge, UK). In control experiments, primary antibodies were omitted and no fluorescence signals were detected.

Results

Transient over-expression of SOX11 in HEK293 cells results in specific gene expression changes as assessed by microarray

Unsupervised hierarchical clustering of gene expression profiles using centred correlation and average linkage revealed that human *SOX11* and control transfection samples have distinct profiles (Fig. 1). This was an indication of both the consistency of the experimental samples used and also the quality of the array hybridisation and detection procedures.

https://doi.org/10.1111/j.1601-5215.2011.00583.x Published online by Cambridge University Press

Fig. 1. Dendrogram of control and *SOX11*-transfected HEK293 cell samples. Hierarchical clustering based on microarray data from each sample reveals two distinct clusters corresponding to *SOX11*-transfected (H1-3AVG) and control cells (L1-3AVG), using centred correlation and average linkage. Thus, *SOX11* transfection induces a reproducible and global change in gene expression.

Identification and validation of SOX11-regulated genes

Limma and *BRB-Array Tools* approaches used in the microarray data analysis generated highly similar results at the levels of rank, fold change and p-value.

A total of 932 genes were significant by SAM analysis (Table S1). The estimated false discovery rate among the 932 significant genes was set at 0.01. Supervised analyses were next performed to identify gene expression differences between human *SOX11* transfected and untransfected HEK293 cells, at a significance level of $p < 0.01$. A total of 251 genes with expression levels altered by at least 1.5-fold were selected for further analysis. Sixty-three of these genes changed by at least two-fold are shown in Table 1.

Confirmation of SOX11 microarray findings at the transcriptional and protein levels and in a second cell line

A panel of eight representative genes (*NDP, NEDD9, HIST1H2BE, HIST1H2AE, SEMA3, BAG1, TUBB3, FILIP1, CYP39A, SCG2* and *CD24*) with a robust fold change in microarray data (Fig. 2a) were chosen for validation using QPCR (Fig. 2b) on the same RNA as used in the microarray experiment.

To determine if SOX11 regulates similar targets in an alternative cell line, SH-SY5Y cells were transiently transfected with the SOX11-expressing construct. Western blot analysis was carried out on two proteins, SCG2 and TUBB3, which displayed up-regulation in the microarray analysis. Both showed clear up-regulation of expression in comparison to ‘empty vector’ or ‘no DNA’ control transfections when normalised to YWHAZ protein loading control (Fig. 2c).

Kinetics of SOX11 activity: time-course QPCR

Total RNA isolated from SOX11 transfected and control cells was used in QPCR assays to assess the kinetics of up-regulation of selected genes from the microarray as this would provide information on how quickly SOX11 acts on target gene promoters (or perhaps if some of the targets were indirectly/secondarily regulated). Harvesting of RNA was carried out at 0, 3, 6, 12, 24 (the time point at which microarray analysis was performed) and 48 h post-transfection. We compared the temporal changes in expression of several up-regulated genes *FILIP1, CYP39A1*, *SEMA3B*, *SCG2*, *GSTA2* and *TUBB3,* relative to control genes (Fig. 3). Comparing the fold changes of these genes revealed a dynamic set of expression profiles that fell into three sequentially and transiently up-regulated groups: (a) *CYP39A1* and *TUBB3* were increased at 6 h and reached their peaks by 24 h, (b) *GSTA2*, *SCG2* and *SEMA3B* were up-regulated by 12 h and (c) *FILIP1* was up-regulated and reached its peak at 24 h.

Ingenuity pathway analysis of SOX11 microarray data

Ingenuity pathway analysis (IPA) was used to identify canonical networks and biological functions/gene ontologies over-represented within the list of 251 SOX11 up-regulated genes with a fold change of more than 1.5. Several neurogenesis-relevant functions and neurological diseases were over-represented in the data set (Table 2) including cell cycle, cell death, cell signalling, cell-to-cell signalling and interaction, cell growth and proliferation, gene expression (regulation), and lipid metabolism. Eighteen SOX11 up-regulated genes were also shown to be involved in nervous system development and function with roles in chemotaxis, growth cone collapse, familial encephalopathy with neuroserpin inclusion bodies, transformation, senescence, cell death and leakage.

Histone and zinc finger genes and chromosomal domain regulation by SOX11

Strikingly, 13 histone genes were up-regulated by the over-expression of SOX11. Closer inspection showed that many were present within a cluster on chromosome 6. We therefore reassessed the microarray fold change data in relation to gene chromosomal coordinates and observed apparent clustering of up-regulated genes in several areas, with chromosomes 6p22.2 and 19q13.43 showing the most robust findings (Fig. 4a–c). This suggested that the transcriptional effects of SOX11 might be indirect in some circumstances: mediated through the alteration of regional chromatin state rather than by direct

https://doi.org/10.1111/j.1601-5215.2011.00583.x Published online by Cambridge University Press

Table 1. SOX11 up-regulated genes with fold change more than two

Unique ID	GB accession number	Gene symbol	Description	Fold change
ILMN_17533	NM_015687	*FILIP1*	Filamin A interacting protein 1 (FILIP1), mRNA	6.25
ILMN_30163	NM_003543	*HIST1H4H*	Histone 1, H4h (HIST1H4H), mRNA	5.26
ILMN_6771	NM_152742	*GPC2*	Glypican 2 (cerebroglycan) (GPC2), mRNA	4.17
ILMN_22946	NM_000266	*NDP*	Norrie disease (pseudoglioma) (NDP), mRNA	4.00
ILMN_28723	NM_013230	*CD24*	CD24 antigen (small cell lung carcinoma cluster 4 antigen) (CD24), mRNA	4.00
ILMN_17622	NM_138720	*HIST1H2BD*	Histone 1, H2bd (HIST1H2BD), transcript variant 2, mRNA	3.70
ILMN_19059	NM_052913	*KIAA1913*	KIAA1913 (KIAA1913), mRNA	2.94
ILMN_1003	NM_005767	*P2RY5*	Purinergic receptor P2Y, G-protein coupled, 5 (P2RY5), mRNA	2.94
ILMN_22069	NM_003548	*HIST2H4*	Histone 2, H4 (HIST2H4), mRNA	2.86
ILMN_24012	NM_016593	*CYP39A1*	Cytochrome P450, family 39, subfamily A, polypeptide 1 (CYP39A1), mRNA	2.86
ILMN_7731	NM_001013672	*LOC400566*	Hypothetical gene supported by AK128660 (LOC400566), mRNA	2.86
ILMN_5522	NM_001031692	*LRRC17*	Leucine-rich repeat containing 17 (LRRC17), transcript variant 1, mRNA	2.70
ILMN_650	NM_182492	*LRP5L*	Low-density lipoprotein receptor-related protein 5-like (LRP5L), mRNA	2.70
ILMN_16399	NM_006086	*TUBB3*	Tubulin, beta 3 (TUBB3), mRNA	2.70
ILMN_18421	NM_182909	*DOC1*	Down-regulated in ovarian cancer 1 (DOC1), transcript variant 1, mRNA	2.63
ILMN_28977	NM_014890	*DOC1*	Down-regulated in ovarian cancer 1 (DOC1), transcript variant 2, mRNA	2.56
ILMN_19455	NM_174896	*C1orf162*	Chromosome 1 open reading frame 162 (C1orf162), mRNA	2.50
ILMN_692	NM_003545	*HIST1H4E*	Histone 1, H4e (HIST1H4E), mRNA	2.50
ILMN_27652	NM_004071	*CLK1*	CDC-like kinase 1 (CLK1), transcript variant 1, mRNA	2.50
ILMN_12847	NM_025231	*ZNF435*	Zinc finger protein 435 (ZNF435), mRNA	2.44
ILMN_4727	NM_018351	*FGD6*	FYVE, RhoGEF and PH domain containing 6 (FGD6), mRNA	2.33
ILMN_7731	NM_001013672	*LOC400566*	Hypothetical gene supported by AK128660 (LOC400566), mRNA	2.33
ILMN_22440	NM_152490	*B3GALNT2*	UDP-GalNAc:betaGlcNAc beta 1,3-galactosaminyltransferase, polypeptide 2 (B3GALNT2), mRNA	2.33
ILMN_10151	NM_006299	*ZNF193*	Zinc finger protein 193 (ZNF193), mRNA	2.33
ILMN_588	NM_006550	*FSBP*	Fibrinogen silencer binding protein (FSBP), mRNA	2.33
ILMN_24579	NM_144661	*C10orf82*	Chromosome 10 open reading frame 82 (C10orf82), mRNA	2.27
ILMN_18282	NM_005319	*HIST1H1C*	Histone 1, H1c (HIST1H1C), mRNA	2.27
ILMN_11057	NM_002896	*RBM4*	RNA binding motif protein 4 (RBM4), mRNA	2.22
ILMN_15870	NM_021908	*ST7*	Suppression of tumorigenicity 7 (ST7), transcript variant b, mRNA	2.22
ILMN_17949	NM_181427	*GABPB2*	GA binding protein transcription factor, beta subunit 2 (GABPB2), transcript variant gamma-3, mRNA	2.22
ILMN_21089	NM_003518	*HIST1H2BG*	Histone 1, H2bg (HIST1H2BG), mRNA	2.22
ILMN_22535	NM_007149	*ZNF184*	Zinc finger protein 184 (Kruppel-like) (ZNF184), mRNA	2.22
ILMN_25666	NM_001005914	*SEMA3B*	Sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3B (SEMA3B), transcript variant 2, mRNA	2.17
ILMN_28905	NM_006417	*IFI44*	Interferon-induced protein 44 (IFI44), mRNA	2.17
ILMN_22538	NM_020801	*ARRDC3*	Arrestin domain containing 3 (ARRDC3), mRNA	2.17
ILMN_19687	NM_198329	*UBE1DC1*	Ubiquitin-activating enzyme E1-domain containing 1 (UBE1DC1), transcript variant 2, mRNA	2.17
ILMN_11266	NM_182597	*FLJ39575*	Hypothetical protein FLJ39575 (FLJ39575), mRNA	2.17
ILMN_16129	NM_018153	*ANTXR1*	Anthrax toxin receptor 1 (ANTXR1), transcript variant 3, mRNA	2.17
ILMN_25145	NM_014969	*WDR47*	WD repeat domain 47 (WDR47), mRNA	2.17
ILMN_10688	NM_014037	*SLC6A16*	Solute carrier family 6, member 16 (SLC6A16), mRNA	2.13
ILMN_1395	NM_002849	*PTPRR*	Protein tyrosine phosphatase, receptor type, R (PTPRR), transcript variant 1, mRNA	2.13
ILMN_11563	NM_032875	*FBXL20*	F-box and leucine-rich repeat protein 20 (FBXL20), mRNA	2.13
ILMN_17827	NM_003469	*SCG2*	Secretogranin II (chromogranin C) (SCG2), mRNA	2.13
ILMN_18286	NM_170662	*CBLB*	Cas-Br-M (murine) ecotropic retroviral transforming sequence b (CBLB), mRNA	2.13
ILMN_21620	NM_134428	*RFX3*	Regulatory factor X, 3 (influences HLA class II expression) (RFX3), transcript variant 2, mRNA	2.13
ILMN_7089	NM_005025	*SERPINI1*	Serpin peptidase inhibitor, clade I (neuroserpin), member 1 (SERPINI1), mRNA	2.08
ILMN_8765	NM_177422	*EIF2C3*	Eukaryotic translation initiation factor 2C, 3 (EIF2C3), transcript variant 2, mRNA	2.08
ILMN_29976	NM_006286	*TFDP2*	Transcription factor Dp-2 (E2F dimerization partner 2) (TFDP2), mRNA	2.08
ILMN_21620	NM_134428	*RFX3*	Regulatory factor X, 3 (influences HLA class II expression) (RFX3), transcript variant 2, mRNA	2.08
ILMN_14828	NM_031421	*TTC25*	Tetratricopeptide repeat domain 25 (TTC25), mRNA	2.08
ILMN_7829	NM_002557	*OVGP1*	*Homo sapiens* oviductal glycoprotein 1, 120 kDa (mucin 9, oviductin) (OVGP1), mRNA	2.08
ILMN_15139	NM_000846	*GSTA2*	Glutathione S-transferase A2 (GSTA2), mRNA	2.04
ILMN_16141	NM_152909	*ZNF548*	Zinc finger protein 548 (ZNF548), mRNA	2.04
ILMN_25508	NM_206919	*ARL9*	ADP-ribosylation factor-like 9 (ARL9), mRNA	2.04

https://doi.org/10.1111/j.1601-5215.2011.00583.x Published online by Cambridge University Press

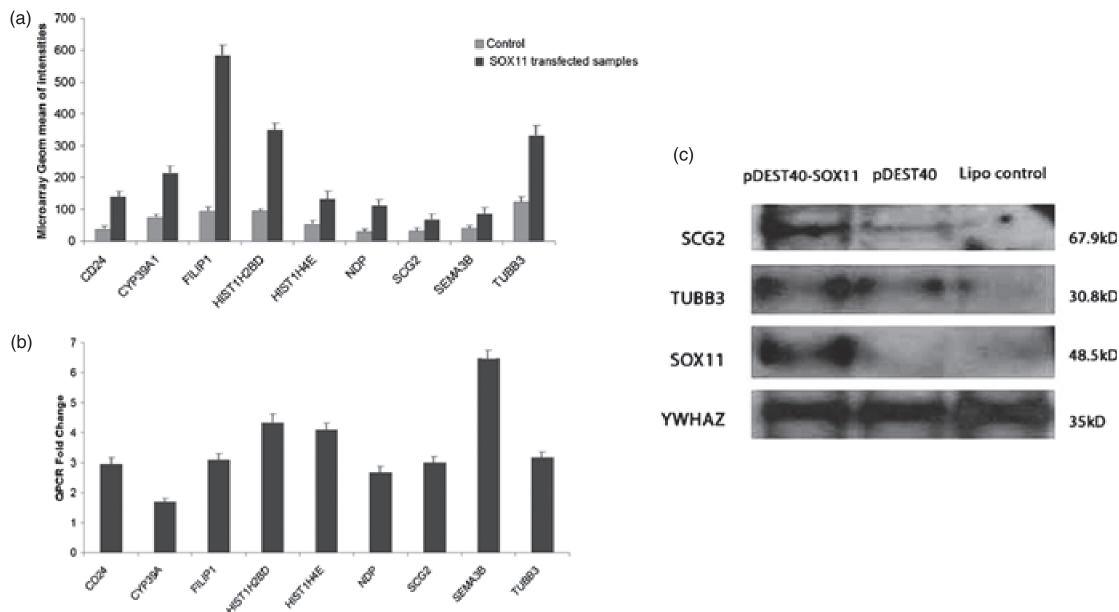

Fig. 2. QPCR (quantitative reverse transcriptase PCR) and Western blotting verify microarray findings. Expression of *CD24, CYP39A1, FILIP1, HIST14E, HIST1H2BD, NDP, SCG2, SEMA3B*, and *TUBB3* determined by microarray (a) and QPCR (b). (a) Microarray signal intensities are expressed as an average of cDNA triplicates from HEK293 cells treated with Lipofectamine (as control) and cDNA triplicates from SOX11-over-expressing HEK293 cells. (b) Fold change values in the QPCR figure were calculated from triplicated analysis of the same HEK293 RNA used in the microarray assays. (c) SH-SY5Y cells were transiently transfected with plasmids pDEST40-*SOX11* or empty pDEST40, or a Lipofectamine-alone control and cultured for 48 h. YWHAZ was used as a reference protein. The protein levels of TUBB3 and SCG2 were significantly up-regulated in cells over-expressing SOX11 protein. The low level of these proteins in the control SH-SY5Y cells suggests that these two proteins may be markers of more differentiated neurons.

promoter effects. Some circumstantial evidence for this is provided by the bell-shaped distribution of SOX11 histone gene up-regulation in the chromosome 6 cluster suggesting a spreading and attenuating influence from a central regulatory point. The subset of genes that are present within these two clusters are listed in Table S3. Most of them are members of histone or zinc finger families.

The expression patterns of Sox11 and an up-regulated protein in the dentate gyrus of the mouse hippocampus

We examined the distribution of Sox11 and a regulated protein in the dentate gyrus of mouse brain – the site of adult neurogenesis. Sox11 was found to be expressed in many brain regions including the whole dentate gyrus, in accordance with the literature. We next tested the distribution of Gpc2, a SOX11 up-regulated gene, and found it localised to the inner, subgranular zone of dentate gyrus with much lower expression in other regions of dentate gyrus (Fig. 5).

Discussion

We have carried out a cell line-based over-expression study coupled with microarray analysis to characterise the set of genes regulated by SOX11 and potentially critical for neurodevelopmental processes important in health and disease. This hypothesis is supported by the microarray data that reveals several genes with known involvement in adult and developmental neurogenesis as well as genes with established links to neuropsychiatric illness (summarised in Table 3).

Although experiments were carried out in non-neuronal cells, we consider it as a biologically and technically valid approach. First, we avoided the confounding effects of developmental homeostatic mechanisms and the transcriptional complexity resulting from multiple cell types that would be faced in studies of brain tissue from, for example, a knockout mouse model. Second, HEK293 cells show expression of many neuronal genes and we identified induced expression of genes typically thought largely as neuron-specific (e.g. *TUBB3*). Third, the use of SOX2 to induce pluripotency from varied somatic cell types strongly suggests that SOX transcriptional targets can be dominantly regulated regardless of cell state.

Analysis of relative fold change in gene expression at different time points after over-expression of SOX11 revealed differing activation kinetics. The different expression profiles of SOX11-regulated genes may indicate the presence of different modes

https://doi.org/10.1111/j.1601-5215.2011.00583.x Published online by Cambridge University Press

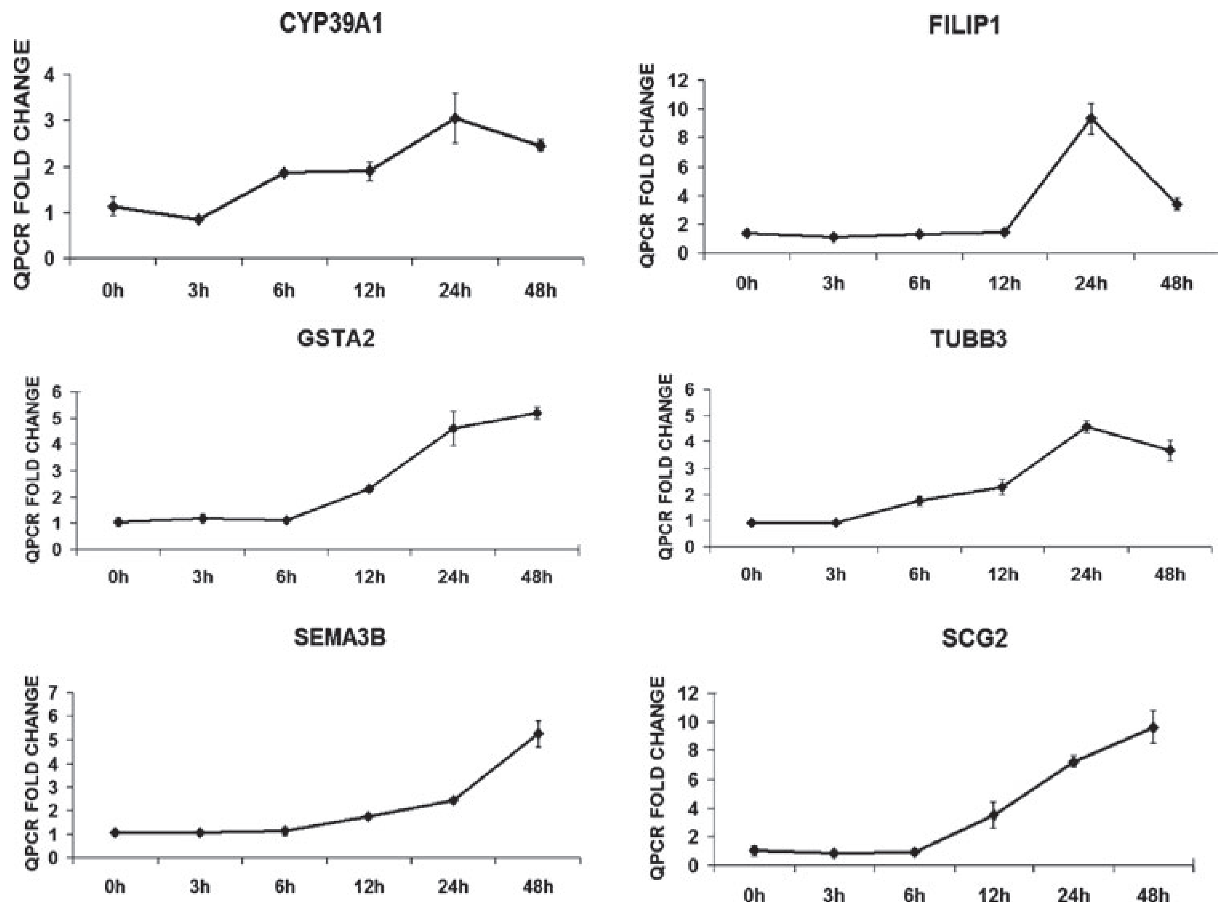

Fig. 3. Time-course quantitative reverse transcriptase PCR of HEK293 cells transiently transfected with *SOX11*. RNA obtained at different time point (from 0 to 48 h) after HEK293 cells was transiently transfected with *SOX11*. Each time point represents fold change (mean ± SD of assay triplicates) in comparison to gene expression in HEK293 cells transfected by control plasmid (pDEST40).

Table 2. Functions and diseases associated with SOX11 targets as determined by ingenuity pathway analysis

Relevant functions and diseases	Relevant gene symbol	Molecules
Cell cycle	*CDKN2B, CDKN2C, COMMD5, HOXA10, IL8, IL1A, ING4, POLB, PPM1D, PPP1R15A* (includes *EG:23645*), *PPP2R2A, SKIL, TFDP2, CXCL12, IVNS1ABP*	16
Cell death	*CXCL12, IL8, IL1A, MCL1, TUBB3, CDKN2C, ANTXR1, POLB*	9
Cell signalling	*CBLB, IL8*	2
Cell-to-cell signalling and interaction	*AKT2, CBLB, CD24, CXCL12, IL8, IL1A, NME1, PPP1R15A 9* (includes *EG:23645), PLXNB1, NEDD9, GJC2, SEMA3B*	12
Cellular growth and proliferation	*CXCL12, IL8, PBX1, MSI2, CBLB*	6
Developmental disorder	*PHF6, PBX1, HOXA10, TGIF1*	5
Gene expression	*AKT2, IHPK2, IL8, IL1A, SOCS4, GABRB2, EIF5, HOXA10, NME1*	10
Lipid metabolism	*CYP39A1, PROX1, AKR1C3, PLA2G4C* (includes *EG:8605*), *IL1A, CXCL12, ETNK1, IL8*	8
Nervous system development and function	*CD24, CXCL12, DPF1, DPYSL4, MAP2, NDP, PCDHB12* (includes *EG:56124*), *POLB, ST8SIA4, TNRC4, GJC2, CHN1* (includes *EG:1123*), *PLXNB1, SEMA3B, DLG2*	18
Neurological disease	*CXCL12, IL8, SERPINI1, HOXA10, CDKN2B*	5
Visual system development and function	*CXCL12*	2

of action or a transcriptional cascade. For example, SOX11 may bind to a promoter region directly or through the alteration of local chromatin states (21) or regulate downstream transcription factors.

Sox11 is expressed in the subventricular zone of lateral ventricles and the subgranular zone of the dentate gyrus. Specifically, Sox11 is strictly localised in Doublecortin (Dcx)-expressing neuronal precursors and immature neurons, but not Sox2-expressing cells (22). Dcx is an intermediate neurogenesis marker expressed in newly formed neurons between the proliferation and final maturation stages (23).

https://doi.org/10.1111/j.1601-5215.2011.00583.x Published online by Cambridge University Press

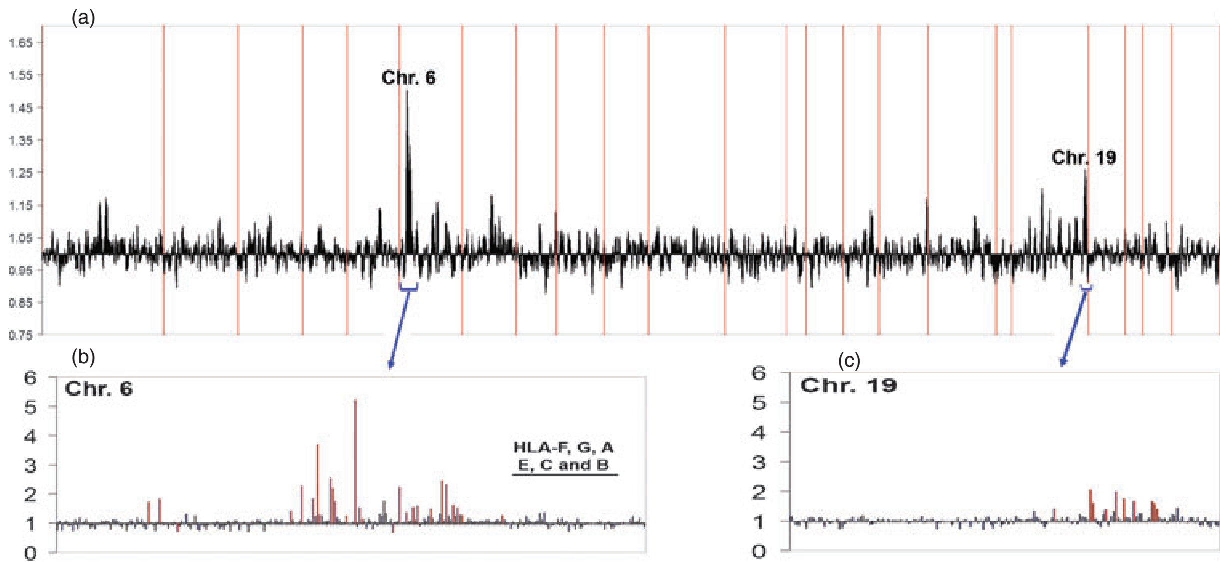

Fig. 4. Clustering of SOX11-regulated genes. Average fold changes in a sliding window of 30 genes along the length of all chromosomes were calculated and plotted (top). Strong peaks indicating clustered up-regulation of genes were observed in chromosomes 6 and 19 (below). In the lower figures, red bars indicate statistically significant gene up-regulation (predominantly histone/zinc finger genes) as determined in the significance analysis of microarray analysis. The horizontal black bar indicates the approximate location of the HLA gene cluster.

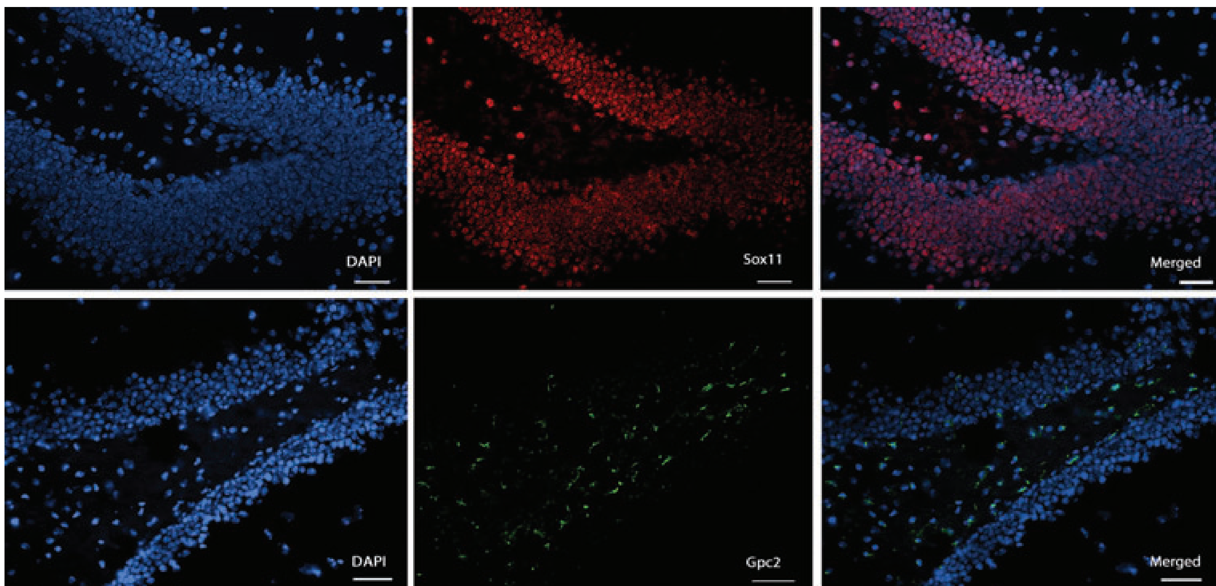

Fig. 5. Sox11 and Gpc2 expression patterns in the dentate gyrus region of mouse hippocampus. Sox11 and Gpc2 protein expression was examined using immunofluorescence on frozen mouse brain sections. Sox11 shows a nuclear distribution in all dentate gyrus granule cells. Gpc2 expression is highly enriched in the subgranular zone where neurogenesis occurs. Localisation is often seen in short axonal projections towards the CA3 region.

This temporal profile of Sox11 activity matches that of the Npas3 transcription factor protein implicated in mental illness (24), which we have shown negatively regulates Sox11 expression (Sha et al., submitted).

At least two clusters of SOX11 targets exist, located on chromosomes 6 and 19. While it might be the case that gene duplication events in these clusters have produced multiple SOX11-regulated genes, the existence of non-histone/zinc finger genes listed in Table S3 suggests that SOX11 exerts its control over the region through indirect means. It has been previously suggested that SOX genes can act by altering chromatin conformation (25). This may be compatible with a regulatory model whereby all genes within a given ‘loop’ of chromosomal DNA are affected by the local chromatin state and its control. The

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Table 3. SOX11 target genes with known functions relevant to neurogenesis and/or neuropsychiatric disorders

Gene	Fold change	Activity	Role
FILIP1	6.25	Filamin A interacting protein	Regulation of neocortical cell migration from the ventricular proliferative zone during development (28)
GPC2	4.17	Heparan sulphate glycoprotein	Expressed in axonal growth cones (29)
CD24	4.00	Glycosylphosphatidylinositol-anchored membrane protein	Regulation of B-cell development and neurogenesis (30,31). Marker for neurogenesis in the adult brain (32,33)
TUBB3	2.70	Beta-3 tubulin component of microtubules	Marker for differentiating neurons (34). Known SOX11 target gene (6,35). Axon guidance role in a human central nervous system syndrome with behavioural phenotypes (36)
ST7 (RAY1/FAM4A1)	2.22	Protein function unknown	Disrupted by a translocation breakpoint in a patient diagnosed with autism (37,38)
SCG2	2.13	Secretogranin II/Chromogranin B. Cell surface sialoglycoprotein. Cleaved into active peptides such as Secretoneurin	Promotes the differentiation of neuroblastoma cells into neurons (39), the outgrowth of immature cerebellar granule cells (40). Up-regulation of human *SCG2* mRNA in dorsolateral prefrontal cortex in schizophrenia (41). Biomarker for schizophrenia in cerebrospinal fluid (42).

zinc finger genes are often transcription factors themselves, whereas the histone genes regulate chromatin state; together these targets may be responsible for a co-ordinated programme of genome regulation promoting neuronal fate commitment and neuronal gene expression. Recent genome-wide association studies of schizophrenia have identified the strongest susceptibility signal over the chromosome 6 cluster. Four of the most significant markers, single nucleotide polymorphisms *rs6913660*, *rs13219354*, *rs6932590* and *rs13211507*, are found within our SOX11-regulated cluster (26,27). This association has been interpreted to indicate a role for the nearby HLA genes in disease risk (Fig. 4b). However, we propose that the histone and zinc finger genes, as mediators of SOX11-regulated programme of neuronal differentiation, are equally credible disease candidates.

Acknowledgements

This research was funded by the Royal Society of Physicians in Edinburgh Sim Fellowship to B. S. P. and a China Council Studentship to L. S. The authors declare that no financial or other conflicts of interest will arise as a consequence of being named as an author in this article.

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Supporting Information

The following Supporting information is available for this article:

Table S1. 932 genes significant by SAM – Significance analysis of microarrays

Table S2. PCR primers used in validation and timecourse QPCR assay. Primers were designed using PRIMER3 [http://workbench.sdsc.edu]. Specificity of all primers was checked by Blast and BLAT analyses http://www.ncbi.nlm.nih.gov/Blast.cgi

Table S3. Significantly (SAM) regulated genes, shown in order, that are found within clusters on chromosomes 6 and 19. The two genes highlighted in bold show downregulation, the rest are upregulated. Histones and zinc finger proteins comprise the majority of the list

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