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ORIGINAL ARTICLE

The long non-coding RNA Gomafu is acutely regulated in response to neuronal activation and involved in schizophrenia-associated alternative splicing

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Schizophrenia (SZ) is a complex disease characterized by impaired neuronal functioning. Although defective alternative splicing has been linked to SZ, the molecular mechanisms responsible are unknown. Additionally, there is limited understanding of the early transcriptomic responses to neuronal activation. Here, we profile these transcriptomic responses and show that long non-coding RNAs (lncRNAs) are dynamically regulated by neuronal activation, including acute downregulation of the lncRNA Gomafu, previously implicated in brain and retinal development. Moreover, we demonstrate that Gomafu binds directly to the splicing factors QKI and SRSF1 (serine/arginine-rich splicing factor 1) and dysregulation of Gomafu leads to alternative splicing patterns that resemble those observed in SZ for the archetypal SZ-associated genes *DISC1* and *ERBB4*. Finally, we show that Gomafu is downregulated in post-mortem cortical gray matter from the superior temporal gyrus in SZ. These results functionally link activity-regulated lncRNAs and alternative splicing in neuronal function and suggest that their dysregulation may contribute to neurological disorders.

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INTRODUCTION

Schizophrenia (SZ) disorder, a debilitating mental disorder affecting about 1% of the population,[1] is believed to be the result of combinations of genetic and environmental factors resulting in brain dysfunction.[2] Despite a high level of heritability observed in SZ,[3] exome sequencing has uncovered only a small percentage of disease-causing genetic alterations associated with the etiology of the disorder.[4,5] Therefore, an understanding of other regulatory mechanisms involved in SZ may explain the 'missing heritability' of the disorder.

It is well accepted that alternative splicing has a role in SZ pathology.[6] Many of the archetypal genes associated with SZ are aberrantly spliced, including disrupted in schizophrenia 1 (DISC1)[7] and v-erb-a erythroblastic leukemia viral oncogene homolog 4 (ERBB4).[8] However, the molecular mechanism underpinning this aberrant alternative splicing is unknown, which precludes the development of rationally designed therapies. Here, we present data demonstrating that the long non-coding RNA (lncRNA) Gomafu (also known as MIAT and RNCR2), which has recently been shown to be associated with alternative splicing through its interaction with splicing factor 1 (SF1),[9] binds several additional nuclear splicing factor proteins, and is involved in alternative splicing of the SZ pathology-related genes *DISC1* and *ERBB4*.

LncRNAs are arbitrarily defined as transcripts longer than 200 bp that possess no protein-coding capacity.[10] Conservative annotations currently estimate >9500 independent lncRNA genes producing >15 500 transcripts from regions antisense, overlapping or intronic to protein-coding genes, or from bidirectional promoters or completely intergenic.[11–13] Recent studies have indicated that lncRNAs may act as modular scaffolds, combining multiple nucleic acid and protein binding domains to direct the localization and/or regulate the activity of multiple effector proteins simultaneously.[14,15] They are involved in multiple processes critical to normal cellular function, including the regulation of gene expression,[15] cell proliferation and differentiation,[16] and disease.[17] The lncRNA Gomafu is localized to a novel nuclear compartment, which is enriched in pre-mRNA splicing factors,[18] and its dysfunction is associated with risk of myocardial infarction.[19] Additional studies have demonstrated roles for Gomafu in retinal cell development,[20–22] brain development[23] and post-mitotic neuronal function,[24,25] although a mechanistic explanation of these functions has been lacking. Gomafu localizes to specific subset of neurons in adult mice,[25] including the CA1 region of the hippocampus and large cortical neurons suggesting a role in neuronal excitatory transmission. Interestingly, Gomafu has also been shown to be upregulated in

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the nucleus accumbens of cocaine and heroin users,[26,27] a region involved in behavior and addiction,[28] suggesting that dysregulation of Gomafu can influence behavior. Notably, Gomafu is also found in a chromosomal region linked to SZ (22q12.1).[29] Here, we demonstrate that Gomafu is acutely regulated by neuronal activity, and is dysregulated in post-mortem cortex of SZ subjects. Combined with its role in SZ-related alternative splicing, these results implicate Gomafu in SZ pathogenesis.

MATERIALS AND METHODS

Mouse primary neurons

Cortices from embryonic day 14 C57BL/6J mice were isolated and mechanically dissociated (as previously described in Martinowich *et al.*[30] where ~90% of the cells were postmitotic neurons) and plated onto poly-L-ornithine hydrochloride- and fibronectin-coated plates at a density of 10^5 cells per cm^2. Cultures were maintained in serum-free neurobasal medium, supplemented with B27 Supplement, L-glutamine and penicillin/streptomycin (Life Technologies, Brisbane, QLD, Australia).

Depolarization and quantitative RT-PCR

Depolarization (50 mM KCl) was performed at 7 days *in vitro* for mouse primary neuronal cultures and at week 8 for human-induced pluripotent stem cells (hiPSCs). Total RNA was isolated using Trizol as per manufacturer's instructions (Invitrogen, Carlsbad, CA, USA), quantified by NanoDrop (Thermo Scientific, Delaware, ME, USA), DNase treated (Turbo DNase, Life Technologies) and reverse transcribed (Superscript III; Invitrogen). Quantitative RT-PCR (qRT-PCR) was performed using the ViiA 7 Real-Time PCR System (Life Technologies) and SYBR Green master mix (Qiagen, Valencia, CA, USA). The relative mRNA level analysis was done by the ΔΔCt method. All the samples were tested in triplicate from at least three independent replicates. Statistical analyses were performed using a two-tailed unpaired *t*-test. Error bars represent the standard error of the mean. Primers used for qRT-PCR are listed in Supplementary Table 1.

Ncode microarray preparation and analysis

RNA samples from three biological replicates for each condition were reverse transcribed and labeled with Cy3 dye according to standard Agilent protocols and hybridized for 18 h to an NCode array (Invitrogen NCRAM-10). Arrays were scanned on an Agilent scanner and data were extracted with Agilent Feature Extraction software. Data were background-corrected, normalized both within and between arrays, and differential expression analysis was performed using LIMMA[31–33] by fitting a linear model of the data to the experimental design matrix and then calculating Bayesian statistics (B statistics) adjusted for multiple testing using Benjamini–Hochberg analysis, assuming that 5% of the genes were differentially expressed as a result of activity. Probes were considered as significantly differentially expressed if the B-statistic was >2.2 and the adjusted *P*-value was <0.05. A custom annotation database previously created for the array was used to determine the number of differentially expressed coding and non-coding probes[34] (Supplementary File 1). GO analysis was performed using the Babelomics software suite;[35] figures were created using the R packages ggplot2[36] and VennDiagram[37] (Supplementary File 1).

HiPSC-derived neuronal culture

HiPSC cultures were maintained in feeder-free culture conditions as described in Xu *et al.*[38] Neuronal differentiation was achieved as described in Supplementary method file 1. Mature hiPSC-derived neurons were tested for calcium trafficking competence using the FLIPR^{TETRA} High Throughput Cellular Screening System as previously described.[39]

Human proteome microarray

Elucidation of Gomafu protein binding partners was conducted using a human proteome microarray as detailed in Jeong *et al.*[40] and analyzed using GENEPIX PRO 5.0 (Molecular Devices, Sunnyvale, CA, USA) that was used to scan the chip images. For every spot, signal intensity was defined as foreground median intensity divided by its local background median intensity. Two rounds of normalization were applied. First, within-chip normalization was used to adjust the median signal intensity of each block to 1. Second, whole chip normalization was applied to standardize this set of signal intensities to a mean of 0 and standard deviation of 1, with the resulting normalized signal intensity called the *z*-score. Arrays contained two identical probes corresponding to each protein and were repeated three times. To be considered as a positive interaction, both probes corresponding to a protein were required to have a *z*-score of $\geqslant 3$ ($P<0.0015$) in at least 2 of the three array experiments conducted, which resulted in 88 positive hits. Median *z*-score for the six conducted replicates is presented in Figure 3a as a kernel density plot. Gomafu probes were synthesized as depicted in Supplementary Figure 1. Briefly, two probes were synthesized from mouse genomic DNA. One probe contained the repeat sequence 'ACUAACC' (Probe A) and the other lacked the sequence (Probe B). These two probes were assayed on the human protein microarray to determine the protein hits specific to the repeat region. Additionally, a human probe was synthesized that uses the endogenous sequence with three repeat sequences within the probe. A graphical representation of median *z*-scores (Supplementary file 2) for significant protein hits using the human probe is shown in Figure 3a.

Electromobility shift assay

Gomafu plasmid was linearized and *in vitro* transcribed using the Promega Riboprobe *in vitro* transcription kit (Promega, Madison, WI, USA). Reactions were spiked with 5 μl of 50 μCi of α-^{32}P CTP and incubated at 37 °C for 4 h. Cy5-labeled riboprobes were purified using 50:50 phenol:chloroform and ethanol precipitated using 5 M NaCl. Electromobility shift assay analysis was conducted as previously reported.[41] RNA probes were incubated with 5, 10 or 20 μg of QKI protein.

RNA immunoprecipitation

SH-SY5Y cells (10×10^6 cells) were lyzed in 1 ml of polysome lysis buffer (10 mM HEPES (pH 7.0), 100 mM KCl, 5 mM $MgCl_2$, 0.5% NP-40, 1 mM DTT), RNAse OUT (Invitrogen) protease inhibitor cocktail (Roche Diagnostics, Castle Hill, NSW, Australia), Vanadyl Ribonucleoside Complexes (New England Biolabs, Beverly, MA, USA). In all, 100 μl lysate was added to 10 μg antibody (human quaking homolog, Novus Biologicals, Littleton, CO, USA; serine/arginine-rich SF 1 (SRSF1), GenWay Biotech, San Diego, CA, USA) and rotated overnight at 4 °C. In all, 100 μl of Dynabeads Protein G (Life Technologies) was added to the antibody/lysate mix, rotated for 2 h at 4 °C, separated using a magnet and washed five times with 1 ml cold NT2 buffer (50 mM Tris (pH 7.4), 150 mM NaCl, 1 mM $MgCl_2$, 0.05% NP-40, 20 μg ml^{-1} Proteinase K). The beads were resuspended in 100 μl NT2 buffer and incubated at 55 °C for 30 min, before being separated using a magnet and the supernatant removed. They were resuspended in 75 μl of elution buffer and rotated at 1000 r.p.m. for 10 min at 37 °C. The beads were then separated using a magnet and supernatant containing the RNA was transferred to a new tube.

Gomafu overexpression in hiPSC-derived neuronal cultures

Overexpression studies were performed using full-length human Gomafu.[9] HiPSC-derived neurons were plated 2–5 days before on-plate nucleofection with the Lonza 4D-Nucleofector system (Lonza Australia, Mt. Waverley, VIC, Australia). Overexpression of Gomafu was performed using 15 μg pCAG-Gomafu per well and 2.0×10^5 cells per well in supplemented AD1-Nucleofector Y solution as per manufacturer's instructions (Lonza Australia). Transfection efficiency was in excess of 50% as quantified by fluorescence-activated cell sorting analysis after 24 h. RNA was collected 72 h later (as described above).

Gomafu knockdown in hiPSC-derived neuronal cultures

Gomafu transcript knockdown was performed using antisense oligonucleotides (ASOs) developed by Isis Pharmaceuticals (Carlsbad, CA, USA) in matured hiPSC-derived neurons, derived as described above. These ASOs are 20-mer oligonucleotides containing a phosphorothioate backbone, 2′-O-(-2-methoxy) ethyl modifications on the first and last five nucleotides and a stretch of ten DNAs in the center. ASOs base pair with their target RNA and cause degradation through the action of RNase H. A 10-μM final concentration of one of three different ASOs targeting Gomafu, and 10 μM of a control ASO with no predicted targets, were added without transfection reagent. ASO-mediated knockdown of Gomafu transcript was confirmed by qRT-PCR after 6 days.

Dissection and extraction of post-mortem tissue samples

Fresh-frozen cortical gray matter from the superior temporal gyrus (STG) was obtained post mortem from 28 subjects with SZ and 28

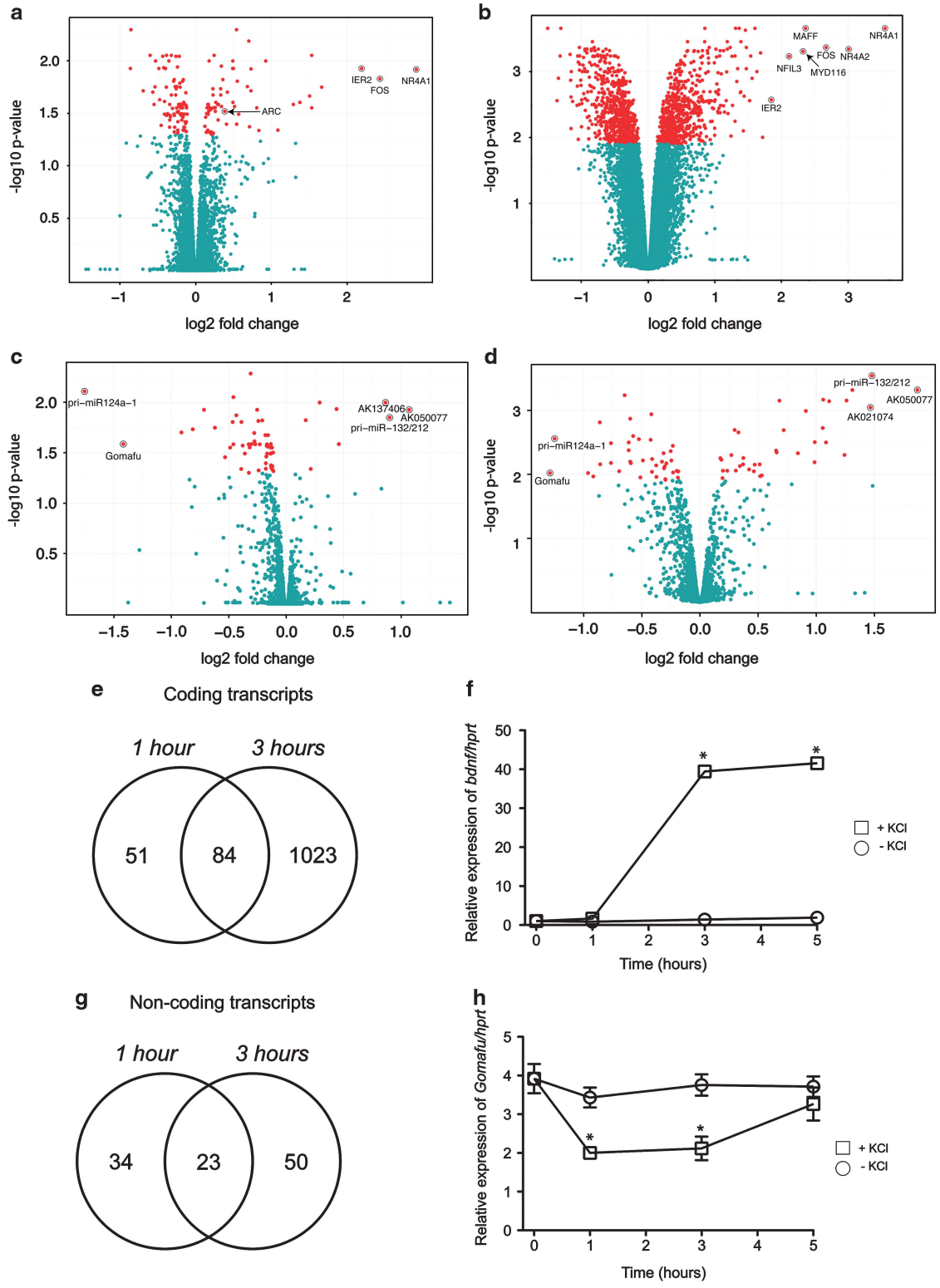

Figure 1. Dynamic expression of coding and non-coding genes in activated mouse primary cortical neurons. Volcano plots reveal significant (red points: B-statistic > 2.2; adj. *P*-value < 0.05) changes in coding gene expression after 1 (**a**) and 3 (**b**) hours of KCl-induced depolarization compared with mock-treated neurons. Expected upregulation of immediate early genes (IEGs) such as *Arc*, *Ier2*, *Fos* and *Nr4a1*, as well as genes such as *Nfil3*, *Ier2*, *Maff* and *Ppp1r15a* (also known as *Myd116* and *Gadd34*), is observed 1 h post activation. Non-coding genes were also differentially expressed both 1 (**c**) and 3 (**d**) hours post activation, with both pri-miR124a-1 and pri-miR-132/212 demonstrating the expected modulation. Other functionally unknown long non-coding RNAs (lncRNAs) were also differentially expressed, such as AK137406, AK050077 and AK021074, while Gomafu transcript was significantly downregulated at both time points. (**e**) The number of significantly altered coding genes increased substantially between 1 and 3 h post activation. (**f**) Quantitative RT-PCR analysis shows the expected upregulation of *Bdnf* expression in depolarized mouse primary neurons. (**g**) The numbers of non-coding transcripts remain relatively constant between 1 and 3 h after stimulation. (**h**) Quantitative RT-PCR analysis reveals that Gomafu expression decreases initially but returns to normal levels within 5 h post activation of mouse primary neurons. *$P < 0.05$.

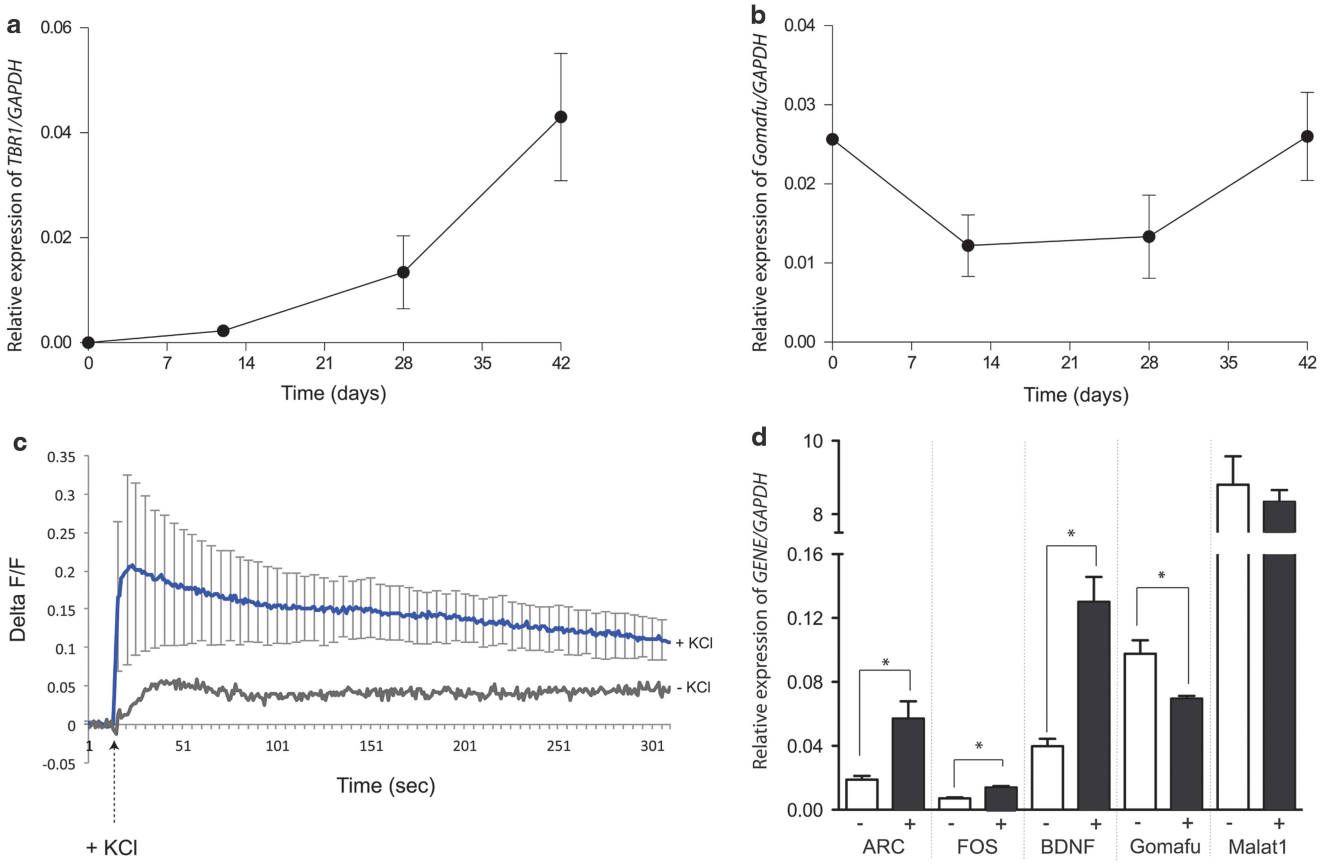

Figure 2. Human-induced pluripotent stem cell (HiPSC)-derived neurons are functional and recapitulate normal physiological gene expression changes upon activation. (**a**) Quantitative RT-PCR analysis demonstrates a significant increase in *TBR1* expression during the final stages of neuronal maturation. (**b**) Quantitative RT-PCR analysis reveals robust Gomafu expression in mature neurons. (**c**) Following differentiation, matured neurons effectively trafficked intracellular calcium upon KCl-induced activation. (**d**) Gene expression changes 3 h post KCl stimulation. Expected changes in immediate early genes (IEGs) such as *ARC*, *FOS* and *BDNF* were observed. Additionally, a significant downregulation of Gomafu, but not MALAT1, was detected in hiPSCs following stimulation. $^*P < 0.05$.

non-psychiatric controls through the NSW Tissue Resource Centre, The University of Sydney, Australia. The gray matter was dissected from the outer edge of blocks of STG tissue from the most caudal coronal brain slice containing the STG (Brodmann's Area 22) as described previously.[42] Dissections were performed blind on coded tissue blocks such that disease status was not identifiable during this procedure. In all cases, a diagnosis of SZ in accordance with DSM-IV criteria was confirmed by medical file review using the Item Group Checklist of the Schedules for Clinical Assessment in Neuropsychiatry and the Diagnostic Instrument for Brain Studies. Consent was obtained from the next of kin and subjects with a significant history of drug or alcohol abuse, or other condition or gross neuropathology that might have influenced agonal state were excluded. In addition, control subjects were excluded if there was a history of alcoholism or suicide. All subjects were of Caucasian descent. Subjects with SZ were matched with controls for gender, age, brain hemisphere, post-mortem interval and pH. The tissue was dissected and RNA extracted as previously described.[43] Relative expression levels of Gomafu were carried out using qRT-PCR (detailed in supplementary method file 2).

Human tissue expression

The Ambion First Choice Human Total RNA Survey Panel (Invitrogen) was used to investigate Gomafu expression in 20 different normal human tissues. Both cDNA preparation and qRT-PCR analysis were performed as described above.

RESULTS

To investigate early neuronal transcriptional changes in response to activity, we depolarized mouse primary cortical neurons with 50 mM KCl for either 1 or 3 h, respectively. RNA was prepared from the KCl- and mock-treated cultures and hybridized to mouse Ncode arrays. These arrays contain probes targeting 19 829 coding and 7504 non-coding features. Expression data are presented in Figures 1a–d, with significantly differentially expressed features (B-statistic >2.2; adjusted P-value <0.05) shown in red. As evidence that depolarization by KCl elicited a typical activity-dependent transcription response, we noted upregulation of classical coding immediate early genes (IEGs) including *Nr4a1*, *Fos* and *Arc* (Lyons and West[44]; Figures 1a and b; Supplementary Table 1). We also noted a significant downregulation in both the 1 and 3 h non-coding RNA data sets of pre-microRNA (miR)-124 (Figures 1c and d), which is one of the few characterized ncRNAs involved in plasticity, and has been reported to be rapidly downregulated in multiple other neuronal activation studies (reviewed in Bredy *et al.*[45]). Gene ontology analysis (Supplementary File 1) demonstrated a significant enrichment of exosomal and RNA-binding proteins, including Pumilio2, a translational repressor known to affect mouse behavior and propensity to seizures,[46] Raver1, a cytoskeletal-associated alternative splicing regulator crucial for synaptic plasticity,[47] and CPEB-1, a regulator of translation localized in the growth cones of developing hippocampal neurons involved in growth hormone-mediated synaptic plasticity.[48,49] These observations provided high confidence that results generated by this system were reflective of normal neuronal depolarization.

Interestingly, although significantly altered coding genes increased 8.2-fold from 1 (135 genes) to 3 h (1107) post

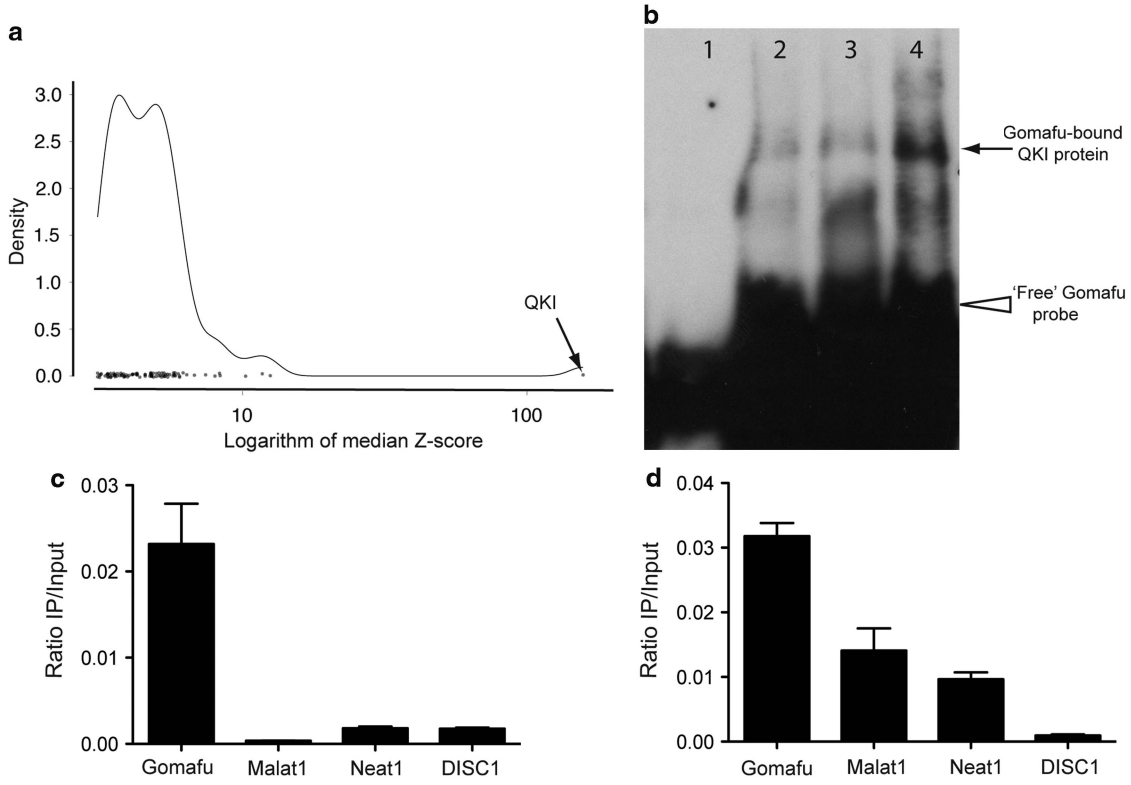

Figure 3. Gomafu binds directly to the splicing factors QKI and serine/arginine-rich SF 1 (SRSF1). (**a**) The kernel density plot of the median *z*-score of three experimental replicate protein microarray results indicates that QKI shows the highest probability association with the repeat containing region of Gomafu out of the proteins assayed. Median *z*-score values for individual proteins are presented as points on the x axis. (**b**) Gomafu interacts with QKI by electromobility shift assay (EMSA) analysis. Lane 1: Gomafu probe alone, lanes 2–4: probe with 5, 10 and 20 μg of human QKI protein. Arrow indicates Gomafu bound to QKI protein; arrowhead indicates 'free' probe. (**c**) RNA immunoprecipitation (IP) demonstrated strong binding of QKI protein to Gomafu RNA, evaluated using qRT-PCR analysis, and minor enrichment of MALAT1, *NEAT1* and *DISC1*. (**d**) Similarly, RNA IP demonstrated robust enrichment of SRSF1 protein to Gomafu RNA with lower enrichment for MALAT1 and NEAT1 and negligible binding to *DISC1*.

stimulation, the number of altered non-coding transcripts remained relatively constant (~1.5-fold increase; Figure 1e). Analysis of activity-regulated genes revealed that 5.8% of coding genes assayed were differentially expressed during neuronal activity but only 1.42% of non-coding genes interrogated were differentially expressed. This correlates well with studies showing high tissue and biological paradigm specificity of lncRNAs.[24,50] One of the most strongly downregulated non-coding transcripts at both time points was Gomafu (Figures 1c and d; Supplementary Table 1). Gomafu is enriched in the brain (Supplementary Figure 2 and Sone *et al.*[25]) and is expressed in discrete populations of post-mitotic neurons in the adult mouse.[25] A neuronal stimulation time course using mouse primary cortical neurons revealed a significant decrease in Gomafu transcript at 1 and 3 h and a return to normal levels by 5 h (Figure 1h).

We next investigated whether the dynamic regulation of Gomafu observed in mouse neurons also occurred in human neurons. To this end, we differentiated hiPSCs into mature neurons. During this *in vitro* neural developmental paradigm, we found a significant upregulation of cortical neuronal markers such as the mature cortical neuronal marker T-box, brain 1 (*TBR1*; Englund *et al.*[51]; Figure 2a), suggesting that iPSC-derived neurons correspond to cortical brain regions previously demonstrated to express Gomafu in mice.[25] In addition, we found that Gomafu is highly expressed in matured iPSC-derived neurons (Figure 2b), implying that these neurons are a suitable model system to investigate Gomafu-related functions in human neurons *in vitro*. We next confirmed that the matured neurons were functional by an intracellular calcium mobilization assay (Fluorescent Imaging Plate Reader (FLIPR) technology; Figure 2c) and qRT-PCR analysis of IEG induction following KCl addition (Figure 2d). Similar to what was observed in primary mouse neurons, a significant down-regulation of Gomafu transcript in hiPSC-derived neurons was observed in response to depolarization (Figure 2d), while MALAT1 (also known as NEAT2), a highly expressed lncRNA also present in discrete nuclear compartments,[52] remained unchanged.

Gomafu is involved in aspects of eye[22] and brain[23] development and post-mitotic neuronal function[24,25] and has recently been shown to bind SF1 through tandem repeats present in exon 5.[9] However, its expression pattern in post-mitotic neurons in distinct regions of the brain[25] suggests additional unknown roles. To further investigate Gomafu-associated protein binding partners, we utilized a human proteome microarray,[40] consisting of over 16 000 unique full-length human proteins. Oligonucleotide probes corresponding to the repeat region in exon 5 revealed additional candidate splicing factors binding to this region (Supplementary Figure 1).

The splicing regulator quaking homolog (QKI) was identified as interacting most strongly with a Cy3-labeled RNA probe derived from the Gomafu repeat sequence (Figure 3a). This binding was validated using electromobility shift assay (Figure 3b) and RNA immunoprecipitation (Figure 3c). Direct binding of SRSF1, shown to bind MALAT1,[53] was also confirmed using a separate candidate approach (Figure 3d). Collectively, these data implicate Gomafu as a possible scaffold for splicing factors, a mechanism recently proposed for MALAT1.[25,53] Intriguingly, QKI has been proposed to be involved in SZ[54,55] and its expression is decreased in specific brain regions in SZ.[56,57] These data, together with a proposed link

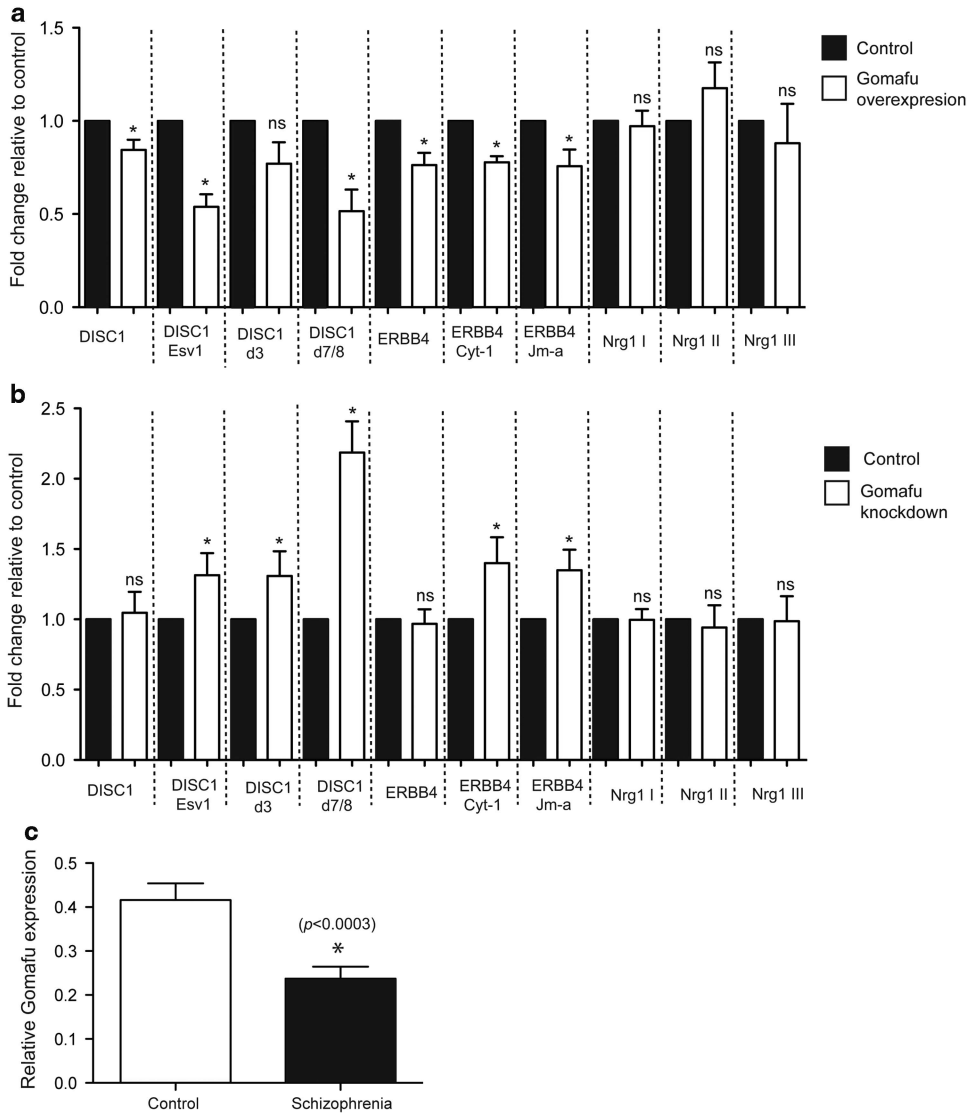

Figure 4. Manipulation of Gomafu expression alters splicing patterns of DISC1 and ERBB4. (**a**) Gomafu overexpression results in significant downregulation of common *DISC1* and *ERBB4* transcripts and concomitant decreases in respective schizophrenia (SZ)-related splice variants of both genes. (**b**) Antisense oligonucleotide (ASO)-mediated knockdown of Gomafu transcript resulted in the upregulation of SZ-related splice variants of *DISC1* and *ERBB4* while the common forms of the transcripts were unaffected. (**c**) Gomafu expression is significantly downregulated in the superior temporal gyrus (STG) in post-mortem cortex of SZ subjects relative to controls ($n = 28$ control subjects; $n = 28$ SZ-affected subjects). *$P < 0.05$.

for the chromosomal region encompassing Gomafu, 22q12.1, with eye movement disorder in patients with SZ,[29] prompted us to determine whether Gomafu might have a role in SZ-related splicing pathology.

It is well established that aberrant alternative splicing of genes including *DISC1*[7] and *ERBB4*[8] has a role in SZ-related pathology.[6] To determine whether Gomafu might be involved in regulating splicing of these genes, we overexpressed and knocked down Gomafu in hiPSC-derived neurons (Supplementary Figure 3A). Indeed, overexpression of Gomafu resulted in a significant decrease in expression of both *DISC1* and *ERBB4* and their alternatively spliced variants (Figure 4a), opposite to the upregulated expression of these splice variants seen in SZ patient brains. Moreover, ASO-mediated knockdown of Gomafu conversely increased the expression of both *DISC1* and *ERBB4* splice variants, but not their unspliced transcripts, almost exactly matching the aberrant splicing pattern seen in post-mortem SZ patient brains (Nakata *et al.*[7] and Law *et al.*[8]; Figure 4b). As both overexpression and ASO-mediated knockdown of Gomafu resulted in no change in *neuregulin 1* (*NRG1*) splice variants previously demonstrated to be differentially expressed in SZ,[58] it seems that there are also likely to be Gomafu-independent pathways involved in SZ-related splicing events. We further extended these studies to investigate SZ-related candidate members of the dopamine (*DRD2*), GABA (*GABRB2*) and glutamate (*GRM3*) pathways[6] and found that *DRD2* splice-variant expression was also reciprocally regulated by Gomafu knockdown and overexpression, while *GABRB2* was not (Supplementary Figure 4). The common and SZ-related forms of *GRM3* were upregulated by Gomafu knockdown, but unaffected by Gomafu overexpression.

Overall, these results were consistent with the exciting possibility that loss of function mutations or decreased expression of Gomafu is involved in driving aberrant cortical splicing patterns observed in SZ post-mortem brains. To further test this hypothesis, we compared the expression levels of Gomafu in post-mortem brains of a cohort of patients that were affected with SZ, and a cohort of unaffected individuals. We found that cortical Gomafu was significantly reduced by 1.75-fold in a cohort of post-mortem

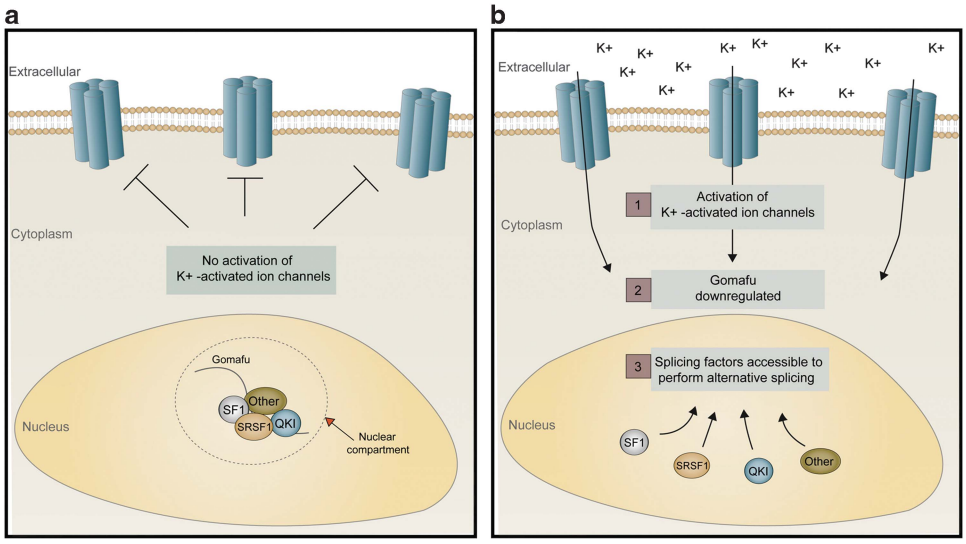

Figure 5. Proposed model for Gomafu-associated alternative splicing. (**a**) In an inactivated neuron, Gomafu exists in nuclear compartments with multiple splicing factors bound. (**b**) Upon neuronal activation, Gomafu expression is downregulated, allowing splicing factors to freely direct the alternative splicing of schizophrenia (SZ)-associated gene transcripts in the nucleus. SF1, splicing factor 1; SRSF1, serine/arginine-rich SF1.

STG in cases relative to controls (Figure 4c), further supporting a potential role for Gomafu in SZ pathogenesis.

DISCUSSION

Disruptions in the DISC1 locus result in higher susceptibility to mental disorders[59] due to its critical roles in regulating neuronal proliferation, migration[60] and synaptic function.[61,62] ERBB4, a receptor tyrosine-protein kinase that is part of the neuregulin pathway, is also strongly implicated in mental illness[63] and is known to undergo alternative splicing producing functionally distinct isoforms.[64] Overexpression of splice variants of both DISC1[7] and ERBB4[8] is associated with SZ; however, the mechanisms responsible for this are unknown. Here, we present data demonstrating that the lncRNA Gomafu, which resides in an SZ-related locus, is dysregulated in SZ patient brains and may contribute to pathogenic splicing of these key genes. In addition, Gomafu may have a constitutive role in regulating plasticity-related activity-dependent alternative splicing.

MiRNAs are strongly linked with neuronal activity and psychiatric disorders such as SZ.[65–68] However, involvement of other classes of non-coding RNA in these processes has not yet been reported. LncRNAs are attractive candidates for involvement in neuronal function as they are highly expressed in the brain in a region and cell type-specific manner, and have been shown to direct generic epigenetic machinery to their target loci[69] thereby affecting epigenetic modifications that are involved in learning and memory.[70] Our data demonstrate that a subset of lncRNAs are dynamically regulated following neuronal activation, and are thus likely to regulate activity-dependent neuronal processes. Moreover, at least one, Gomafu has a role in SZ-related alternative splicing, implicating dysregulation of lncRNAs in psychiatric disease. We also show for the first time that Gomafu binds to QKI, a protein itself implicated in SZ,[54,55] suggesting that Gomafu-related pathways may contribute to the pathophysiology of SZ.

Gomafu is known to be highly enriched within neurons in the brain, and to bind multiple splicing factors. Our data now suggest a functional model of Gomafu in neurons that includes regulation of neuronal activity-dependent alternative splicing, possibly by acting as a splicing factor scaffold that allows splicing factor release upon transient downregulation following depolarization (Figure 5). Such a mechanism seems quite plausible, given that two other lncRNAs, TUG1 and MALAT1, have been shown to function as protein scaffolds by binding to the methylated and unmethylated form of Polycomb 2 protein, respectively, and serving as scaffolds for the assembly of corepressor/coactivator complexes.[71] The dynamic nature of this mechanism would allow splicing of neuronal genes to be tightly and acutely controlled in response to activation. Dysregulation of such a mechanism may result in a defective neuronal network as is present in SZ. Interestingly, MALAT1 was the first lncRNA proposed to function as a 'splicing factor sponge'[53] and although shown to be involved in synaptogenesis[52] its expression remained unchanged in our model of acute neuronal activation. This could imply that lncRNA function is restricted to particular neuronal processes, serving as parts of sophisticated regulatory control systems.

This study reveals that lncRNAs are differentially expressed in neuronal activation, and we uncover one activity-dependent lncRNA, Gomafu, that is associated with activation and pathological SZ-related alternative splicing. Moreover, we show that Gomafu is dysregulated in a cohort of post-mortem cortical tissue from SZ subjects, further suggesting a molecular pathway with potential relevance to the pathophysiology of SZ. These data open up new avenues for psychiatric disease research and potential therapeutic design targeting lncRNA.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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