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Transcriptional Factor 4 Association with Schizophrenia

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Abstract

Schizophrenia (SCZ) is a highly heritable, severe, common psychotic disorder. Genetic variants play an important role in SCZ pathogenesis and progression. Preclinical and clinical findings, particularly in the genome-wide association studies (GWAS), have identified transcriptional factor 4 (TCF4, E2-2) as a high risk gene for SCZ. Disruption of TCF4 expression and function has been also linked to Pitt-Hopkins syndrome (PTHS). TCF4 is well known to regulate neuronal lineage differentiation during embryonic and adult neurogenesis. Recent studies have identified a novel role of Tcf4 in regulating the neurite branching/pruning and synaptic plasticity of postmitotic differentiated neurons. Further understanding of the cellular and molecular mechanisms underlying the neurodevelopmental disorders and neuropsychological/neuropsychiatric diseases associated with TCF4 deficiency or dysfunction will provide important translational targets for the treatment of SCZ and PTHS.

Keywords: Schizophrenia; TCF4; CRISPR-Cas9; Neurons; Neurogenesis

Introduction

Schizophrenia (SCZ) is a major life-long psychiatric disorder, affecting men and women equally in 1% of the general population. The majority of SCZ patients remain ill after the initial episode, suffering from chronic and severely incapacitating symptoms. Genetic studies identified as high as 80% heritability of this complex and devastating mental disease [1-3]. Genetic variants contribute much to the pathogenesis and progression of SCZ. More and more genome-wide association studies (GWAS) have identified transcription factor 4 (TCF4) as a high risk gene for SCZ [2,4-6]. A CTG triplet repeat expansion in an intronic region of the TCF4 gene is associated with SCZ [7,8]. Six single nucleotide polymorphisms (SNPs) of TCF4 [rs12966547 (G) P=2.6 × 10^{-10}, rs9960767 (C) P=4.1 × 10^{-9}, rs4309482 (A) P=7.8 × 10^{-9}, rs10401120 (T), rs2958182 (T) and rs17512836 (C) P=2.35 × 10^{-8}] have been identified as risk variants for SCZ [9-12]. In the Han Chinese population, three TCF4 SNPs (rs9320010, rs7235757, and rs1452787) are also significantly related to the risk of SCZ [2]. The findings in recent GWAS from psychiatric GWAS consortium (PGC) with 36,989 cases and 113,075 controls further support TCF4's role in genetic susceptibility to SCZ [13]. However, no significant association between rare TCF4 sequence variants and SCZ is found [14]. Among the TCF4 variants, rs12966547 and rs8766 are associated with the age at onset (AAO), which is a known prognostic indicator for SCZ [11]. Disruption of TCF4 expression and function has been also linked to several other common and rare diseases such as Pitt-Hopkins syndrome (PTHS), Fuchs' endothelial corneal dystrophy (FECD) [15,16], and primary sclerosing cholangitis (PSC) [6,17]. In particular, PTHS is well known to be caused by *TCF4* haploinsufficiency [18-20], and is characterized with intellectual disability, autism, epileptic seizures, absent speech, hyperventilation and gut dysfunction [21-23]. Therefore, targeting TCF4 by modulating its expression/function might be a highly promising therapeutic to treat these already-diagnosed patients with TCF4 mutations or deficiency.

Extensive Function of TCF4, Particularly in Neurogenesis and Neuronal Polarization

TCF4 is also known as E2-2; ITF2; PTHS; SEF2; ITF-2; SEF-2; SEF2-1; SEF2-1A; SEF2-1B; SEF2-1D; bHLHb19 (http://www.ncbi.nlm.nih.gov/gene/6925). TCF4 should not be confused with the T-cell factor 4 that regulates Wnt signaling and also associates with SCZ [24,25]. TCF4 is a member of class I basic helix-loop-helix (bHLH) transcription factors. The bHLH family, known as E-proteins due to their binding to Ephrussi box (CANNTG) [18,19], play critical roles in a huge array of cellular processes and organogenesis. TCF4 is extensively expressed in many tissues but enriched in neurogenic niche during embryonic, postnatal and adult neurogenesis [6]. Among the bHLH factors, only TCF4 is continuously expressed in the adult nervous system in both animals and humans [6]. Generally, TCF4 heterodimerizes with class II bHLH proteins, particularly the proneuronal factors such as NeuroD, Neurogenin, ATOH1/MATH1, ASCL1/MASH1 to regulate neurogenesis and neuronal lineage differentiation [26]. TCF4 homodimerizes [27] to regulate gene expression [28,29]. TCF4 also heterodimerizes with class V bHLH proteins to inhibit gene expression [30,31]. These findings suggest that TCF4 plays an important role in regulating embryonic and adult neurogenesis and synaptic plasticity. TCF4 is also involved in lymphoid development [32], epithelial-mesenchymal transition [33,34], ocular growth [35], T cell differentiation [36] and dendritic cell development [37,38].

The expression of class I/II bHLH proteins is required to initiate neuronal differentiation of neural stem cell [39]. However, the class II bHLH proteins play a role in regulating the function of terminally differentiated neural cells. For example, in *Drosophila*, the class II bHLH protein Atonal functions to promote neuronal migration, axon guidance and arborization [40]. In mammals, the Atonal homolog Atoh1/Math1 is essential for migration of postmitotic retrotrapezoid nucleus neurons required for proper respiration [41]. Several class I bHLH proteins are expressed in postmitotic neurons in *C. elegans* [42], mice [43], humans [43], suggesting that class I bHLH proteins may also play an important

role in postmitotic neurons. The daughterless (*Da*) gene encodes the only type I bHLH protein found in *Drosophila* [44]. Similar to TCF4, the Da protein is expressed in a large number of tissues and is involved in a diverse number of developmental processes including neurogenesis [28,45], cell proliferation [46,47], muscle development [48], ovarian development [49], and retinal development [47,50]. Over expression of Da in mitotic epithelial cells is sufficient to arrest these cells in G2 phase of the cell cycle [51]. Our recent studies show that Da is expressed in postmitotic neurons of the fly neuromuscular junction, and functions to restrict axonal arborization (bouton), while Tcf4 is also expressed in mouse postmitotic neurons and functions to restrict neurite branching [52]. However, more experimental evidence is warranted to support the importance of Tcf4/Da in neuronal polarization, in particular the presynaptic (axonal) *vs* the postsynaptic (dendritic) branching/pruning.

Tcf4 Binding Partners and Downstream Target Genes in Postmitotic Neurons

In addition to binding to the target DNA, TCF4 binds to many proteins, particularly bHLH family of transcription factors. Its homodimerization or heterodimerization with binding partners contributes to its action in regulating the expression of target genes [26,28,29]. Bioinformatics analysis identified thousands of downstream target genes that are regulated by TCF4/Da in mitotically active cells [33,53]. For example, TCF4 binds to proneuronal proteins such as NeuroD, neurogenin, Mash1, Math1 to regulate neurogenesis and neuronal lineage differentiation [54-56]. Neurexin 1 (Nrxn1) is emerging as susceptibility factor for SCZ and its rare copy number variants are found to contribute to the pathogenesis of SCZ [57,58]. Deletions within the Nrxn1 gene affecting exons confer SCZ risk and are associated with autism and mental retardation [59,60]. TCF4 directly binds to Nrxn1 promoter/enhancer region in mature mammalian neurons to repress its transcription [52]. In *Drosophila*, Da forms homodimers which mediate synapse restriction via inhibition of Nrxn1 expression [52]. Thus, Nrxn1 may partially mediate TCF4-related phenotypes for SCZ. The variants in miR-137 gene are significantly associated with SCZ. SCZ patients with homozygous miR-137 risk allele display significant decreases in occipital, parietal and temporal lobe gray matter concentration [61]. Dysregulation of miR-137-regulated genes such as TCF4, PTGS2, MAPK1 and MAPK3 may underlie the gray matter loss seen in SCZ patients [61]. TCF4 acts as a regulator of neuronal intrinsic excitability by suppressing two ion channel genes, Kcnq1 and Scn10a [62]. The downstream effectors of TCF4 pathway are essential for understanding the molecular mechanisms underlying TCF4-associated diseases. Altering the expression or function of TCF4 downstream effectors may improve the cognitive in SCZ patients. Potential therapeutic chemical/natural compound(s) could be screened in human cell model or transgenic animal models to control the expression of TCF4 and/or target genes [63]. Recently, HDAC inhibitor treatment in mouse model has been shown to rescue learning and memory deficits caused by loss of TCF4 function [64]. To develop valid therapeutics, controlling specific genes' expression is pivotal.

The Potential Therapeutic Strategies and CRIPSR-Cas9 Technology

So far, no effective treatment is available for TCF4-associated diseases although different therapeutics has been used to improve the SCZ symptoms, such as antipsychotic prescription and psychosocial intervention [65-67]. It is still a big challenge to develop new effective therapy for SCZ because the etiology and pathogenesis of SCZ remain largely unclear. Many transcripts/isoforms exist in both human TCF4 and mouse Tcf4. Different types of cells (particularly subtypes of neurons) may express different isoforms at various levels. Various types of SNPs may contribute to SCZ development in a different way. Therefore, isoform-specific and/or cell-specific manipulation of TCF4 expression and/or gene mutation correction would provide a novel therapy for TCF4-assocaited diseases. Spatiotemporal control of endogenous non-mutant TCF4 in attempt to restore the "normal" expression/function of TCF4 may be also an interesting therapeutic strategy for patients with TCF4 haploinsufficiency.

The RNA-guided endonuclease CRISPR-Cas9 technology has emerged as a simpler and more versatile technology to target and modify any genomic sequence with high levels of efficacy and specificity [68]. Successful application of Cas9 technology to mammalian system for genome editing was first reported in early 2013 [69,70]. Since then, this novel genome editing system has attracted a huge amount of attention in the biomedical field. In particular, extensive preclinical examples have been seen in the fields of animal models, genetic diseases, cancer biology and infectious diseases [71-75]. Efficient Cas9-mediated transgene knockin has been recently reported in various cell lines [76-81] and different species including mice [82,83], rats [84,85], pigs [86,87], zebrafish [81,88,89], etc. Simultaneously, the use of the catalytically-deficient Cas9 (dCas9) conjugated with a single transcriptional activator or repressor to manipulate cellular gene regulation has been developed [90-93]. This single regulator system has its limitations, such as effectiveness of gene activation or repression and scalability. Thus, recruitment of multiplex transcriptional activators or repressors through guide RNA (gRNA) modification and/or dCas9 fusion has been explored [94-99]. For example, dCas9-based synergistic activation mediator (SAM) system has been developed by engineering the single gRNA (sgRNA) through appending a minimal hairpin aptamer to the tetraloop and stem loop 2 of sgRNA [100]. Such aptamer is capable of binding to the dimerized MS2 bacteriophage coat proteins. Thus, a novel MS2-p65-HSF1 complex guided by target-specific MS2-mediated sgRNA (msgRNAs) could facilitate the potency of dCas9-mediated gene activation by up to 3,000 fold [100]. This dCas9-SAM technology is capable of activating the provirus in HIV-1 latent cells for the "shock and kill" strategy to cure HIV/AIDS [101]. Similarly, a dCas9-engineered transcriptional repressor (ETR) system that combines several epigenome repressors has been established to achieve long-term suppression of endogenous genes [102]. The dCas9 system relies on target-specific sgRNAs and delivers multiple exogenous transcriptional activators or repressors to the target site. Furthermore, the dCas9 does not keep nuclease activity, and never induce any DNA mutation or chromosome translocations in host cells. Therefore this dCas9 system has high specificity, high efficiency and no/low cytotoxicity.

Most exciting application of Cas9 technology is the genetic correction [103-105] and antiviral treatment in animals [106,107] and likely soon in the clinic. Clinical trials for Cas9-mediated cancer targeting have been initiated [108,109]. The proof of concept to apply the Cas9 and/or dCas9 genome/epigenome editing technology to the manipulation of SCZ risk genes has been proposed in several reviews [110-112]. Experimental evidence to target SCZ-derived induced pluripotent stem (iPS) cells has been reported recently [113,114]. The effective knockout of Tcf4 by Cas9/sgRNA in embryonic neural cells via in-utero electroporation has been shown to alter the intrinsic excitability of prefrontal neurons [62]. However, it is still a long journey to unlock genetics of SCZ diseases using CRISPR-Cas9 technology.

The spatiotemporal expression of the *TCF4* gene is extraordinarily intricate and difficult to study because (1) There are many splicing events and variant transcripts of TCF4 in both human and animals, with 30 exons in mouse *Tcf4*; (2) No good antibody against TCF4 (and particularly its various isoforms) is currently available; (3) Traditional knockin tagging (reporter) modeling is time-consuming and insufficient. Even with a good antibody, the immunohistochemistry study cannot detect the dynamic and quantitative changes in TCF4 protein. TCF4 promoter-driven

reporter assay may address the transcriptional regulation of different *TCF4* transcripts in human and animals, but this *in vitro* assay is artificial and does not measure the spatiotemporal expression of endogenous TCF4, rather than various isoforms. Therefore, CRIPSR-Cas9 mediated reporter knockin technology may provide a novel approach to manipulate the spatiotemporal expression of the endogenous TCF4 gene in cultured cells (*in vitro*) or transgenic animal models (*in vivo*). Many drugs have been approved by FDA for their safety and pharmacokinetic profiles in patients. In addition, the manufacturing and distribution networks are readily available. Therefore, repurposing existing drugs for novel unanticipated function would be a rapid and effective way to develop potential new therapy for neurodevelopmental disorders and neuropsychological/neuropsychiatric diseases. The highly sensitive bioluminescence assay in endogenous TCF4 reporter knockin cells may provide an excellent high throughput screening for FDA-approved drugs. By screening, we may identify potential FDA-approved drugs that specifically and efficiently upregulate or downregulate the expression of endogenous TCF4 in human iPS-derived cells or animal models.

Conclusion

In the context of all the TCF4-associated diseases, the critical question is which copy, the insufficient normal TCF4 or the over expressed mutant TCF4, is the *de novo* cause to the development and progression of these diseases. Therefore, better understanding the expression pattern and different function of the mutant and non-mutant TCF4 in the nervous system, cornea and liver is fundamental to the development of novel therapeutic treatment for these debilitating diseases. The causative effect of the haploinsufficient normal TCF4 and the mutant TCF4 due to SNP variants, triplet repeat or deletion has yet to be characterized. Developing a novel dual reporter disease model by targeting endogenous normal and mutant copies of TCF4 might be an important future direction. We expect to see that manipulation of insufficient normal TCF4 expression may counteract the mutant TCF4 to achieve sufficient normal function.

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