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Cognitive and Pharmacological Insights from the Ts65Dn Mouse Model of Down Syndrome

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Abstract

Down syndrome (DS) is a multi-faceted condition resulting in the most common genetic form of intellectual disability. Mouse models of DS, especially the Ts65Dn model, have been pivotal in furthering our understanding of the genetic, molecular and neurobiological mechanisms that underlie learning and memory impairments in DS. Cognitive and pharmacological insights from the Ts65Dn mouse model have led to remarkable translational progress in the development of therapeutic targets and in the emergence of DS clinical trials. Unravelling the pathogenic role of trisomic genes on human chromosome 21 and the genotype-phenotype relationship still remains a pertinent goal for tackling cognitive deficits in DS.

Introduction

Trisomy of human chromosome 21 (Hsa21) causes overexpression of more than 500 genes, resulting in the multi-faceted genetic condition characterised as Down syndrome (DS) [1,2]. With an incidence of approximately one in 650-1000 live births worldwide, DS is the most common genetic form of intellectual disability [3]. Accelerated and precocious aging occurs in DS, as does early-onset Alzheimer's disease (AD), which is manifested in over 75% of people with DS by the age of 65 [3-5]. Learning and memory impairments in DS are marked by perturbed neurodevelopment, altered neuronal structure, and synaptic plasticity deficits. The cognitive profiles in DS vary in both expressivity and severity; conceivably from allelic differences in Hsa21 genes and the complex interplay with other non-Hsa21 genes, epigenetic influences and environmental factors. Understanding these genotype-phenotype

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correlations may help develop pharmacological interventions. Mouse models of DS, including the Ts65Dn mouse, recapitulate many cognitive phenotypes of DS and have been instrumental in elucidating the molecular pathogenesis underlying DS, mapping Hsa21 genes to various phenotypes, and assessing the effect of potential therapeutic targets [6-8]. Herein, we highlight recent insights obtained from the Ts65Dn mouse model to unravel mechanisms of learning and memory impairments in DS; and how these findings have led to recent breakthroughs in pharmacological interventions.

Cognitive insights from the Ts65Dn mouse

Neurodevelopment

Neurodevelopment is perturbed in DS as demonstrated by a reduced brain volume, reduced number of neurons, and abnormal neuronal morphology in several brain regions; particularly the granule cells in the cerebellar cortex [9]. Compared to healthy infants, brains of DS infants show an increase in total dendritic branching and higher total dendritic length, which then steadily decreases to lower than normal levels during adolescence and into adulthood. These structural and dendritic differences may contribute to perturbed cortical information processing and decreased synaptic plasticity [10]. It is proposed that elongation of the cell cycle length, from decreased Sonic hedgehog growth factor response, results in reduced proliferation rates, leading to impaired neurogenesis [9]. A deficient mitotic response to the Sonic hedgehog growth factor in the Ts65Dn mice is proposed to cause the decreased proliferation of the cerebellar granule cells and an alteration in neural crest progenitor cells, which could contribute to the DS-associated craniofacial dysmorphology [11,12]. Cerebellar granule cell deficits in neural progenitor cells as well as an elevated rate of cell death have been documented in other mouse models of DS [13,14]. Oxidative stress levels indicative of elevated rates of neuronal apoptosis are also increased in DS fibroblasts [15,16].

GABAergic system and synaptic plasticity

The majority of the forebrain is comprised of excitatory glutamatergic projection neurons and approximately 10% inhibitory γ-aminobutyric acid (GABA) interneurons. Neuronal development and cognitive functioning is dependent on a balanced ratio of excitatory and inhibitory neurons. A developed and functioning cortex evolves from the neurogenesis of the proper neurotransmission of excitatory and inhibitory neurons, in distinct sites of origin, followed by the migration and differentiation of these neurons within the neocortex [17-19]. Alterations in neuronal morphology, function, and neurotransmission have been proposed to cause synaptic plasticity deficits and impairments in long-term potentiation (LTP), a neural correlate for learning and memory.

Neurophysiological studies in the Ts65Dn mouse have revealed enlarged boutons and dendritic spine heads in cortical and hippocampal neurons and excessive inhibition leading to failed LTP induction in the hippocampus and fascia dentate [20-22]. This increased inhibitory input has been attributed to an altered efficiency of the GABAergic system in the DG of Ts65Dn mice, rather than a decrease in inhibitory synapse density, and is a proposed mechanism for synaptic plasticity defects in DS [21-26]. Electrophysiological data revealed enhanced $GABA_A$ and $GABA_B$ receptor-mediated neurotransmission with an accompanied reduction of paired-pulse ratios of evoked inhibitory postsynaptic currents (IPSCs); suggesting increased presynaptic release of GABA. These data correlate with larger, but not increased, number of inhibitory synapses found in the DG of Ts65Dn mice.

Contribution of Hsa21-encoded genes

The perturbed neurodevelopment and the over-inhibition in DS and Ts65Dn mice is likely caused by triplicated genes on Hsa21 (Table 1). Oligodendrocyte transcription factor 1

(*Olig1)* and lineage transcription factor 2 (*Olig2)* genes are implicated in neurogenesis and oligodendrogenesis [27,28]. Normalising these two genes to disomic levels in Ts65Dn mice corrected the enhanced inhibitory interneuron phenotype, providing a causal explanation of the gene-dosage imbalance of *Olig1* and *Olig2* genes in producing the excitatory-inhibitory (E-I) imbalance [29].

Enhanced postsynaptic $GABA_B$ signalling could be explained by the triplication of the *KCNJ6* (potassium inwardly-rectifying channel, subfamily J, member 6) gene and increased expression of the protein it encodes, Kir3.2, a channel that modulates postsynaptic $GABA_B$ receptors. Overexpression of *Kcnj6* in Ts65Dn mice leads to increased Kir3.2 channel density, increased current, and increased inhibitory $GABA_B$ signalling [30]. A recent study also documented enhanced $GABA_B$/Kir3.2 signalling in DG granule cells of Ts65Dn mice [25]. *Kcnj6* overexpression has also been suggested to lead to an imbalance between $GABA_B$ and $GABA_A$ inhibition of CA1 pyramidal neurons through a pathway specific mechanism to perturb hippocampal circuitry functioning [31]. *Synaptojanin 1* (SYNJ1) encodes a nerve terminal protein that is implicated in membrane trafficking and is another Hsa21 gene that is essential for maintaining GABAergic neurotransmission stability [32].

DYRK1A (dual specificity tyrosine-(Y)-phosphorylation-regulated kinase 1A) is heavily implicated in neurodevelopment and is strongly expressed in neural precursor populations during embryonic neurogenesis. It is conceivable that altered *DYRK1A* expression levels perturb developmental pathways, leading to postnatal neurodevelopmental difficulties [33]. Overexpression of *DYRK1A* has been found to decrease neuron-restrictive silencer factor (REST/NRSF) chromatin remodelling complex levels and to deregulate genes that contribute to DS-associated neuronal phenotypes, including dendritic growth impairments, pluripotency, and embryonic stem cell fate [34,35]. RCAN1 (regulator of calcineurin 1) is a negative regulator of calcineurin that subsequently modulates NMethyl-D-aspartate receptor (NMDAR) activation kinetics by decreasing the probability of opening time of the NMDAR channel. *DYRK1A* directly phosphorylates *RCAN1*, leading to reduced nuclear factor of activated T-cells (NFATc) translocation to the nucleus. NFATc transcription factors are regulators of vertebrate development and destabilisation of this regulatory circuit through triplication of *DYRK1A* and *RCAN1* may contribute to the enhanced Tau phosphorylation seen in DS [36,37].

DSCAM (Down syndrome cell adhesion molecule) has a critical role in dendrite morphology and neuronal wiring. Overexpression of *DSCAM* in hippocampal neurons inhibits dendritic branching; impairments in NMDA-mediated regulation of DSCAM local mRNA translation may be one mechanism through which aberrant dendritic morphology and synaptic plasticity deficits occurs during development [38]. Overexpression of *SIM2* (single-minded homolog 2), a transcriptional repressor, dramatically reduces levels of *DBN1 (Drebrin 1)*, a neuronal gene that modulates dendritic spine cytoskeletal dynamics at postsynaptic terminals. The reduction of DBN1 levels could explain the morphological neuronal changes and the resulting learning and memory deficits prevalent in DS [39,40].

Sod1 (superoxide dismutase 1) overexpression also reduces hippocampal neuronal progenitors and LTP, enhances sensitivity to degeneration and apoptosis, and up-regulates GABAergic neurotransmission [41,42]. Amyloid protein (*APP)* is strongly implicated in neurodegeneration, and triplication of this gene has been associated with early onset AD [43]. However, recent evidence for a role in neurodevelopment for *APP* stems from studies in which lowering of beta-amyloid levels, an APP metabolite that is the main constitute of amyloid plaques in AD, improved cognitive deficits in Ts65Dn mice [44].

Pharmacological insights from the Ts65Dn mouse

The identification of behavioural, morphological, and neurobiological alterations in the Ts65Dn mouse model have led to invaluable insights into the pathogenesis of DS that allow for potential therapeutic targets to be explored (Table 2).

SSRIs and mood stabilisers

Chronic treatment in Ts65Dn mice with fluoxetine, a serotonin selective reuptake inhibitor (SSRI), increased neurogenesis by enhancing the proliferation and survival of neurons in the DG [45]. Recently, studies examined whether early pharmacotherapy with fluoxetine could improve neurogenesis. Untreated Ts65Dn neonatal mice exhibited impaired cellular proliferation and demonstrated normal levels of serotonin (5-HT), but a lower expression of 5-HT1A receptors and brain-derived neurotrophic factor (BDNF) levels [46]. Treating Ts65Dn neonatal mice with fluoxetine not only rescued impaired proliferation and increased the number of surviving cells, but also restored the expression of 5-HT receptors and BDNF levels to that of control mice [46]. Lithium has also been examined as a potential treatment to improve neurogenesis. Treating Ts65Dn mice with lithium restored cellular proliferation in the subventricular zone [47]. These studies demonstrate the potential of early pharmacotherapy to correct for neurogenesis impairments by using readily available and approved drugs.

Neuroprotective peptides

Pharmacological intervention with neuroprotective peptides has also been demonstrated to promote neurodevelopment. Vasoactive intestinal peptide (VIP) levels are altered in DS; and cortical astrocytes in Ts65Dn neonatal mice demonstrate reduced responsiveness to VIP stimulation [48,49]. Activity-dependent neuroprotective protein (ADNP) and activity-dependent neurotrophic factor (ADNF) are neuroprotective neurotrophic factors released by VIP stimulation of astrocytes [50]. Combined treatment of DS cortical neurons with active fragments of ADNP and ADNF, NAPVSIPQ (NAP) and SALLRSIPA (SAL) respectively, increased neuronal survival, restored morphological changes and protected from oxidative damage and apoptosis [51]. The efficacy of these neuroprotective peptides in preventing developmental delay and glial deficits through prenatal treatment was examined in Ts65Dn mice. Untreated Ts65Dn mice displayed developmental delays in achieving motor and sensory milestones, downregulated ADNF expression, and glial deficits [52]. Prenatal treatment with NAP+SAL reversed all these deficits [52]. This study identifies a potential intervention during pregnancy that could improve developmental delays and glial deficits in DS.

$GABA_A$ antagonists

To restore the E-I imbalance, several pharmacological interventions have aimed to decrease the excessive inhibition of GABAergic neurotransmission prevalent in Ts65Dn mice [25,26]. Ts65Dn mice have been treated with non-competitive $GABA_A$ antagonists, pentylenetetrazol (PTZ) and picrotoxin (PTX), which inhibit $GABA_A$ receptors. Chronic treatment with PTZ reversed the deficits seen in the novel object recognition task (NORT) and spontaneous alternation tasks in Ts65Dn mice [53]. Surprisingly, the improvement in cognition and LTP was sustained for up to 2 months after initial treatment, suggesting a lasting effect of treatment on neuronal circuit modification. Chronic treatment with PTZ for 8 weeks in Ts65Dn mice did not modify sensorimotor abilities and locomotor activity in home cages; however it did rescue learning and memory performance in the Morris water maze (MWM) task [54]. Treating Ts65Dn mice with PTX also reversed deficits in NORT that were exhibited in untreated mice; these improvements were retained for up to 2 weeks [53]. In untreated Ts65Dn mice, impaired LTP was coupled with reduced synaptic activation

of NMDAR due to excessive inhibition of DG cells [21]. Administering PTX to suppress inhibition resulted in improved induction of LTP and normalised NMDAR-mediated currents [21]. Recently, chronic treatment in Ts65Dn mice with an inverse agonist selective for the α5 subunit of the $GABA_A$ benzodiazepine receptor (α5IA) improved cognitive deficits in the MWM and normalised *Sod1* overexpression with an enhancement in learning-evoked immediate early genes expression levels [55]. Encouraged by this body of evidence, Roche, a healthcare company, recently announced the commencement of a trial to examine the cognitive impact of reducing GABAergic neurotransmission in the hippocampus using a drug selective for the α5 subunit of $GABA_A$ receptors [56].

NMDAR antagonists

Learning is also improved by the non-competitive NMDAR antagonist, memantine, which reduces abnormal activation of glutamate neurotransmission. Administration of memantine, an open-channel antagonist, rescued Ts65Dn performance deficits in a fear conditioning test [57] and improved spatial learning in MWM task [58]. Long-term memantine treatment improved spatial reference memory in a MWM task and recovered object discrimination ability in a NORT, but spontaneous activity remained unaltered [59]. Upon histopathological analysis, no morphological modifications indicative of neuroprotection were observed in the neurons of the basal forebrain or locus coeruleus (LC), however, an increase in BDNF expression was documented in the hippocampus and frontal cortex [59]. Interestingly, acute treatment of memantine 30 mins prior to testing was sufficient to enhance performance on the NORT [59]. Despite mouse studies demonstrating promising benefits of memantine, a recently published clinical trial reported that memantine is not an effective pharmacological treatment for cognitive decline or dementia in people who are above 40 years old and have DS [60]. This suggests that therapies that are effective in people with AD may not necessarily confer benefits in DS.

Conclusion

Triplication of Hsa21 genes leads to a plethora of multi-system pathologies that characterise DS, rendering it complex to understand. Despite this, since the discovery of DS in the 19th century, the life expectancy of people with DS has increased from an average age of 12 years old in the 1940s to 60 years of age at present due to dramatic advances in medical treatment and social intervention [3]. Mouse models of DS, especially the Ts65Dn mouse, have provided an unequivocal contribution to dissecting the genetic, molecular and neurobiological processes that underlie the syndrome, and in deciphering the genotype-phenotype relationship of overexpressed Hsa21 genes in causing the clinical manifestation of DS. This approach has successfully led to the development of pharmacological targets and the emergence of DS clinical trials. However, to fully understand the genetic basis of DS and its consequent perturbations still remains a challenge and further investigations are necessary to tackle various aspects of the syndrome. The development and study of DS mouse models that more closely resemble the gene-dosage imbalance in humans with DS, and genome-wide association studies of individuals with DS, will be instrumental in identifying dosage-sensitive genes and the pathogenic mechanisms underlying DS-associated phenotypes.

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Highlights

- DS causes perturbed synaptic plasticity and excessive inhibitory neurotransmission
- Ts65Dn mouse model recapitulates behavioural and cognitive phenotypes of DS
- Several triplicated Hsa21-associated genes in Ts65Dn mice are implicated
- Insights from Ts65Dn have led to pharmacological interventions and clinical trials

Table 1

Physiological and pathogenic role of affected Hsa21 genes

Hsa21 genes	Physiological role	Pathogenic role	Reference
OLIG1/OLIG2	Implicated in neurogenesis and oligodendrogenesis	Causes GABAergic excitatory-inhibitory imbalance	Lu et al., 2002; Zhou & Anderson, 2002; Chakrabarti et al., 2010
KCNJ6	Effector protein for $GABA_B$ receptors; modulates potassium channel current and density	Contributes to enhanced $GABA_B$ signalling	Best et al., 2007; Best et al., 2011; Kleschevnikov et al., 2012
SYNJ1	Nerve terminal protein implicated in membrane trafficking and synaptic transmission	Inability to maintain stable GABAergic neurotransmission	Luthi et al., 2001
DYRK1A	Regulates signalling and cell proliferation; involved in neurogenesis and neurodevelopment	Deregulates genes implicated in dendritic growth, cell pluripotency and embryonic stem cell fate; deregulates NFAT circuits and may cause enhanced Tau phosphorylation	Altafaj et al., 2001; Lepagnol-Bestel et al., 2009; Canzonetta et al., 2008; Arron et al., 2006; Jung et al., 2011
RCAN1	Inhibits calcineurin-dependent signalling pathways affecting development; modulates NMDAR activation	Decreases opening probability of NMDAR channel; deregulates NFAT circuits and may cause enhanced Tau phosphorylation	Arron et al., 2006; Jung et al., 2011
DSCAM	Cell adhesion molecule with a critical role in dendrite morphology and neuronal wiring	Inhibits dendritic branching and causes aberrant synaptic plasticity; aberrant NMDA-mediated regulation of DSCAM local translation	Alves-Sampaio et al., 2010
SIM2	Transcriptional repressor implicated in synaptic plasticity and morphology	Reduces DBN1 levels causing morphological cytoskeletal changes at postsynaptic terminals in dendritic spines	Ooe et al., 2004; Hayashi et al., 1996
SOD1	Cytoplasmic protein implicated in oxidative stress	Reduces hippocampal neuronal progenitors and LTP; enhances sensitivity to degeneration and apoptosis; up-regulates GABAergic neurotransmission	Gahtan et al., 1998; Levkovitz et al., 1999
APP	Cell surface receptor and transmembrane precursor protein that promotes transcriptional activation	Forms the protein basis of amyloid plaques prevalent in AD and DS; mechanism of action unknown	Rovelet-Lecrux et al., 2006; Netzer et al., 2010

Table 2

Pharmacological interventions to tackle DS-associated cognitive deficits

Neurobiological Pathway	Pharmacological Compound	Cognitive Effect	References
SSRIs and mood stabilisers	Fluoxetine	Increases neurogenesis by enhancing proliferation and survival of neurons; restores 5-HT receptor and BDNF levels	Clark et al., 2006; Bianchi et al., 2010
	Lithium	Increases neurogenesis by restoring cell proliferation in subventricular zone	Bianchi et al., 2010
Neuroprotective peptides	NAPVSIPQ+SALLRSIPA	Protection from oxidative damage and apoptosis; prenatal treatment prevents developmental delays, glial deficits and aberrant ADNF expression	Brenneman et al., 2004; Busciglio et al., 2007; Toso et al., 2008
$GABA_A$ antagonists	Pentylenetetrazol	Improves hippocampal-based learning in behavioural tasks and enhances LTP	Fernandez et al., 2007; Rueda et al., 2008
	Picrotoxin	Improves hippocampal-based learning in behavioural tasks, enhances LTP and normalises NMDAR-mediated currents	Kleschevnikov et al., 2004; Fernandez et al., 2007
	α5IA	Improves hippocampal-based learning in behavioural tasks, normalises Sod1 overexpression and enhances learning-evoked immediate gene expression levels	Braudeau et al., 2001; Roche, 2011
NMDAR antagonists	Memantine	Improves hippocampal-based learning and fear conditioning in behavioural tasks, reduces excessive glutamate neurotransmission and increases BDNF levels	Costa et al., 206; Rueda et al., 2010; Lockrow et al., 2010; Mohan et al., 2009