



# Microglia as possible therapeutic targets for autism spectrum disorders

Megumi Andoh, Yuji Ikegaya, Ryuta Koyama\*

Laboratory of Chemical Pharmacology, Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, Japan

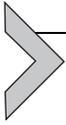
\*Corresponding author: e-mail address: rkoyama@mol.f.u-tokyo.ac.jp

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## Abstract

Malfunions of the nervous and immune systems are now recognized to be fundamental causes of autism spectrum disorders (ASDs). Studies have suggested that the brain's resident immune cells, microglia are possible key players in ASDs. Specifically, deficits in synaptic pruning by microglia may underlie the pathogenesis of ASDs, in which excess synapses are occasionally reported. This idea has driven researchers to investigate causal links between microglial dysfunction and ASDs. In this review, we first introduce the characteristics of microglia in ASD brains and discuss their possible roles in the pathogenesis of ASDs. We also refer to immunomodulatory agents that could be potentially used as symptomatic therapies for ASDs in light of their ability to modify microglial functions. Finally, we will mention a possible strategy to radically cure some of the symptoms reported in ASDs through reorganizing neural circuits *via* microglia-dependent synaptic pruning.



## 1. Introduction

Some decades ago, it was revealed that the synaptic density in the human brain is drastically increased during childhood and decreased during adolescence to the adult level.<sup>1</sup> The decrease in synaptic density has been called synaptic pruning or synapse elimination and has been suggested to be crucial for the normal development of brain functions.<sup>2,3</sup> Accumulating studies have shown that microglia, the brain-resident immune cells, are the executors of synaptic pruning.<sup>2,4</sup> Deficits in synaptic pruning are suggested to perturb refinement of neural circuits, leading to abnormal development of higher-order brain functions such as cognition and social communication, which are the main symptoms of autism spectrum disorders (ASDs), one of the neurodevelopmental diseases.<sup>3,5</sup> Therefore, many researchers have focused on the relationship between ASDs and microglia.

Neuropathological analysis using postmortem brains of ASD patients shows that microglial number is increased in that population and that microglia exhibit the activated form (enlarged cell body and thick process) in multiple regions, including the frontal lobes.<sup>6,7</sup> PET/CT scan analysis also shows that microglia are activated in ASD frontal lobes and cerebellum.<sup>8</sup> Moreover, in the ASD prefrontal cortex (PFC), the microglia-neuron distance is reduced, and microglial processes enclose the neuronal cell body, which suggests that the interaction between microglia and neurons is facilitated in ASD brains.<sup>9</sup> It should be noted, however, that the likely activated status of microglia in ASD brains is reported from studies that analyzed postmortem specimens or individuals who had been already diagnosed with ASDs; whether microglia are activated or even inactivated during the development of ASDs remains to be elucidated.

Recently, the complement molecules C1q and C3, which stimulate the microglial complement receptor CR to prune synapses,<sup>4</sup> have been suggested to be related to ASDs. In the serum of ASD patients, the levels of C1q and C-reactive protein, which promote the inflammatory response through binding to C1q, are elevated.<sup>10,11</sup> An increased concentration of complement molecules in ASDs may enhance microglia-dependent synaptic pruning. It is also possible that excess complements mask the differences in complement deposition levels on each synapse, which may result in the disruption of complement-targeted specific synaptic pruning.

Almost all of the reports from human studies have been based on samples from patients already diagnosed with ASDs or on postmortem brain

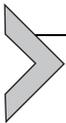
specimens. To investigate microglial contributions to the development of ASDs in detail, animal models of ASDs have been widely used. In maternal immune activation (MIA) models, which mimic maternal infection during pregnancy as a risk factor for ASDs, microglial density and proinflammatory cytokine levels are increased in the cerebral cortex and the hippocampus.<sup>12,13</sup> Neurons also exhibit unusual morphologies in these models. For example, hippocampal granule cells have more spines.<sup>14</sup> Furthermore, the expression levels of CX3CR1, a molecule suggested in neuron–microglia interactions, were decreased in the hippocampus, suggesting that MIA perturbs microglia–dependent synaptic pruning.<sup>14</sup> *Fmr1* KO mice, in which the risk gene for fragile X syndrome is depleted, were also used as a model of ASD and exhibited impaired microglial phagocytosis of postsynaptic protein PSD95.<sup>15</sup> In addition to these primary ASD models, the BTBR mouse strain, which is used as an idiopathic ASD model, has more activated microglia with higher levels of MHC-II compared to the C57BL/6J mouse strain.<sup>16</sup> It has also been revealed that hippocampal pyramidal cells show increased spontaneous excitatory postsynaptic currents (EPSCs) in BTBR mice, suggesting deficits in synaptic pruning.<sup>17</sup>

Some reports have shown that deficits in microglia–dependent synaptic pruning cause ASD-like features. Inhibition of microglial autophagy decreases microglial synaptic phagocytosis, leading to increased spine density and impaired social interaction.<sup>18,19</sup> Mice lacking CX3CR1, which is expressed by microglia and regulates synaptic pruning, exhibited decreased functional connectivity in ASD-related brain regions, impaired social interaction, and increased repetitive behaviors.<sup>20</sup> More recently, *TREM2*, which is a risk gene of Alzheimer’s disease, has been suggested to be necessary for developmental synaptic pruning, and *TREM2* KO mice exhibit reduced functional connectivity in the brain, impaired social interaction and increased repetitive behavior.<sup>21</sup>

The male-to-female ratio of ASD morbidity is approximately 4 to 1 in humans; thus, it is believed that there are relationships between sexes and ASDs. Microglia have attracted attention as a factor that causes sexual differences in ASDs. A genome-wide association study (GWAS) has revealed that genes expressing at higher levels in prenatal human males are also upregulated in postmortem brains of ASD patients and, importantly, these genes include microglial markers.<sup>22</sup> Sex differences in microglial properties are now being investigated energetically. One such study has shown that expression levels of the lysosomal protein CD68 are higher in female mice during development when surplus synapses are eliminated, suggesting that

phagocytic capacity differs between sexes.<sup>23</sup> This finding may suggest an excess of synapses underlying the elevated ASD morbidity rate in males.

Astrocytes are the most abundant glia cells in the brain. They contact adjacent synapses and form the tripartite synapse, modulating synaptogenesis and synaptic plasticity *via* providing and receiving ions and cytokines. These astrocytes' functions suggest their involvement in the pathophysiology of ASDs, which is also referred to as a synaptopathy.<sup>24,25</sup> Indeed, astrocytes are persistently activated in the cerebellum of a mouse model of Fragile X syndrome.<sup>26</sup> However, a report shows that morphology and number of astrocytes do not change in postmortem brains of ASD patients.<sup>27</sup> Furthermore, few studies have investigated astrocyte-related genes and molecules in ASD animal models. Thus, further investigation is necessary to clarify the role of astrocytes in the pathogenesis of ASDs.



## 2. Therapeutic targets for ASD treatment and possible involvement of microglia

To date, two drugs, risperidone and aripiprazole, are approved by the US FDA to be used for the treatment of ASD symptoms.<sup>28</sup> Some reports have suggested that these drugs modulate microglial functions. For example, risperidone inhibits the production of inflammatory cytokines in microglia *in vitro*, while aripiprazole reduces microglial activation in an MIA model mouse.<sup>29,30</sup> In the serum and cerebrospinal fluid (CSF) of ASD patients, inflammatory cytokines such as IL-1 $\beta$  and IL-6 are expressed at higher levels, which implies immunological dysfunction in ASDs.<sup>31</sup> This chapter discusses the use of immunomodulatory agents as a potential therapeutic strategy for ASDs (listed in Table 1). We also introduce how these agents possibly modify microglial functions. In the latter part, we discuss cell therapy and its future prospects.

### 2.1 Drug therapy

#### 2.1.1 Luteolin

Luteolin is a naturally occurring flavonoid. Luteolin decreases the serum level of tumor necrosis factor (TNF) and IL-6 and has been shown to improve the social interaction of ASD patients.<sup>32,33</sup> In an MIA model mouse, luteolin also ameliorates elevated anxiety and impaired sociability.<sup>34</sup> Additionally, BV-2 microglial activation and subsequent neurotoxicity induced by lipopolysaccharide (LPS) are inhibited by luteolin application *in vitro*.<sup>59</sup>

**Table 1** Drug therapies for ASDs.

Drug	Subject species	Administration route		Reference
		Dosage	Finding	
Luteolin	Human ASD patients	Orally 100 mg	Decreased serum levels of IL-6 and TNF Improved communication and social skills	32
	Human ASD patients	Orally 200 mg/10 kg	Improved GI and allergy symptoms Increased social interaction	33
	IL-6-induced ASD model mice	Orally 10 mg/kg/day	Decreased TNF- $\alpha$ and IL-1 $\beta$ in brains Decreased repetitive behavior Improved social interaction	34
	BV-2 microglia <i>in vitro</i>	~50 $\mu$ M	Inhibited proinflammatory pathway Enhanced anti-inflammatory pathway	35
Minocycline	Human ASD patients	50 mg twice/day	Decreased hyperactivity and Irritability	36
	VPA-induced ASD model rats	Orally 25 and 50 mg/kg	Decreased BBB permeability Improved social interaction Decreased repetitive behavior	37
	Microglia prepared from Poly(I:C)- induced ASD model mice <i>in vitro</i>	Orally 3 mg/kg/day	Increased phagocytic capacity	38
Suramin	Human ASD patients	Intravenously 20 mg/kg	Improved social interaction Decreased repetitive behavior	39
	Poly(I:C)-induced ASD model mice	Intraperitoneally 20 mg/kg	Improved social interaction Improved hippocampus-dependent memory	40
Vitamin D	Human ASD patients	Orally 300 IU/kg/day	Improved social interaction Decreased irritability	41
	Mouse microglia <i>in vitro</i>	10 nM	Decreased proinflammatory cytokines Increased anti-inflammatory cytokines	42

*Continued*

**Table 1** Drug therapies for ASDs.—cont'd

<b>Drug</b>	<b>Subject species</b>	<b>Administration route Dosage</b>	<b>Finding</b>	<b>Reference</b>
Gut microbiota	Poly(I:C)-induced ASD model mice	Orally ND	Corrected GI deficits Decreased repetitive and anxiety behavior Improved communicative behavior	43
Immunoglobulin	Human ASD patients	Intravenously 0.4 g/kg	Reduced hyperactivity and irritability Improved communication ability	44
	Mouse microglia <i>in vitro</i>	20 $\mu$ M	Increased A $\beta$ clearance by microglia	45
Pioglitazone	Human ASD patients	Orally 30 mg/day	Decreased hyperactivity and irritability	46
	Human ASD patients	Orally 30 or 60 mg/day	Decreased hyperactivity, irritability and stereotypy	47
Pioglitazone	APP/PS1	Orally $\sim$ 150 mg/kg/day	Increased A $\beta$ phagocytosis by microglia	48
	HAIP microglia	$\sim$ 20 $\mu$ M	Inhibited proinflammatory cytokine expression induced by LPS	49
Sulforaphane	Human ASD patients	Orally 50–150 $\mu$ mol	Reduced hyperactivity, irritability and repetitive behavior Improved communication ability	50
	BV2 microglia Primary mouse microglia	2.5 $\mu$ M	Reduced proinflammatory cytokine expression induced by LPS	51
	N9 microglia	$\sim$ 10 $\mu$ M	Reduced proinflammatory cytokine expression and ROS production induced by LPS	52

Celecoxib	Human ASD patients	Orally 100–300 mg/day	Improved social interaction Decreased irritability and repetitive behavior	53
	Rats	Intraperitoneally 20 mg/kg	Decreased the number of activated microglia in substantia nigra and striatum	54
Lenalidomide	Human ASD patients	Orally 2.5 mg/day	Decreased serum levels of TNF- $\alpha$ Improved social interaction Decreased irritability and repetitive behavior	55
	Parkinson's disease model mice BV-2 microglia	NS 100 mg/kg 250 $\mu$ g/mL	Decreased microgliosis in striatum Reduced proinflammatory cytokine expression induced by $\alpha$ -synuclein	56
Spironolactone	Human ASD patients	Orally 2 mg/kg/day	Decreased irritability, hyperactivity and repetitive behavior	57
	Rats	Intrathecally ND	Suppressed microglial secretion of inflammatory cytokines induced by chronic compression of the dorsal root ganglion	58

ND, not determined; NS, not shown.

### 2.1.2 Minocycline

Minocycline is a tetracycline analog and is widely used to suppress microglial activation, although its mechanism of action remains to be revealed. Minocycline has been suggested as an effective adjuvant of risperidone because patients cotreated with minocycline and risperidone show greater improvements in hyperactivity and irritability than patients treated with risperidone alone.<sup>60</sup> In a valproic acid (VPA)-induced rat model of ASDs, which mimics a drug-induced ASD, minocycline ameliorates ASD-like behaviors and disruption of the blood-brain-barrier (BBB).<sup>37</sup> Loss of BBB integrity is one of the pathological features of ASDs, and it is shown that BBB disruption causes microglial activation *in vivo*.<sup>61,62</sup> Minocycline has also been shown to rescue the decreased phagocytic ability of microglia prepared from MIA model mice *in vitro*.<sup>38</sup> Thus, minocycline possibly normalizes synaptic density in the ASD brain *via* inducing engulfment of surplus synapses by microglia.

### 2.1.3 Suramin

Suramin is a polyanionic compound with an unknown mechanism of action. Intravenous administration of suramin improves social interaction and repetitive behavior in ASD patients.<sup>39</sup> MIA model mice also show improved social interaction and memory after suramin treatment.<sup>40</sup> Suramin inhibits microglial activation in the spinal cord *in vivo*.<sup>63</sup> Additionally, suramin inhibits P2X7-dependent secretion of IL-6, CCL2 and TNF- $\alpha$  from mouse primary microglia.<sup>64</sup> A recent report has shown that P2X7 drives ASD behaviors in MIA model mice.<sup>35</sup> Thus, P2X7 can be a possible therapeutic target for ASDs.

### 2.1.4 Vitamin D

In ASD patients, the serum levels of vitamin D and its receptor are reduced.<sup>36</sup> Vitamin D supplementation ameliorates irritability and impaired social interaction in ASD patients.<sup>41</sup> Vitamin D decreases the expression of inflammatory cytokines such as IL-6 and TNF- $\alpha$ , while it increases the expression of antiinflammatory cytokine IL-10 from mouse primary microglia stimulated by LPS or IFN- $\gamma$ .<sup>42</sup>

### 2.1.5 Gut microbiota

Because many ASD patients suffer from gastrointestinal disturbances, the gut microbiota has been considered.<sup>65</sup> Microbiota transfer therapy improves irritability, communication skills and sociability in ASD patients.<sup>43</sup> The gut microbiota regulates microglial development and homeostasis.<sup>66</sup> For example, microglia in mice without gut microbiota were hyper-ramified

and expressed higher levels of maturation and activation marker, while their immune response to LPS infection was diminished.<sup>66</sup> In a mouse model of MIA-induced ASDs, the composition of microbiota is disrupted, and tight junctions of intestinal mucosa become fragile.<sup>67</sup> Transplantation of human commensal *Bacteroides fragilis* into a mouse model of MIA-induced ASDs improves repetitive and anxiety behaviors.<sup>67</sup>

### **2.1.6 Immunoglobulin**

Intravenous administration of immunoglobulin reduces hyperactivity and irritability and elevates communication ability in ASD patients.<sup>44</sup> Furthermore, IgG has been shown to promote A $\beta$  phagocytosis by murine microglia *in vitro*.<sup>45</sup> Thus, it is possible that IgG induces engulfment of increased synapses by microglia in the ASD brain.

### **2.1.7 Pioglitazone**

Pioglitazone is a thiazolidinedione with antidiabetic properties and potential antineoplastic activity. Oral administration of pioglitazone reduces hyperactivity, irritability and repetitive behaviors in ASD patients.<sup>46,47</sup> Pioglitazone enhances A $\beta$  phagocytosis by microglia in Alzheimer's disease model mice, while it suppresses secretion of IL-6, TNF- $\alpha$ , and iNOS from microglia stimulated by LPS *in vitro*.<sup>48,49</sup> Therefore, pioglitazone could promote engulfment of excess synapses in the ASD brain by microglia without inflammation and neurodegeneration.

### **2.1.8 Sulforaphane**

Sulforaphane is the most characterized isothiocyanate. Oral administration of sulforaphane reduces hyperactivity, irritability and repetitive behaviors in ASD patients in addition to improving their communication ability.<sup>50</sup> Sulforaphane inhibits LPS-induced microglial activation processes, such as proinflammatory cytokines (IL-1 $\beta$ , IL-6 and TNF- $\alpha$ ) expression and reactive oxygen species (iNOS) release, in murine microglial cultures.<sup>51,52</sup>

### **2.1.9 Celecoxib**

Celecoxib is a pyrazole derivative and a selective cyclooxygenase 2 inhibitor. Celecoxib, in combination with risperidone, ameliorates irritability, repetitive behavior and impaired social interaction in ASD patients.<sup>53</sup> The LPS-induced increase in the number of activated microglia is inhibited by celecoxib in neonatal rats.<sup>54</sup>

### 2.1.10 Lenalidomide

Lenalidomide is a phthalimide and piperidone derivative that has immunomodulatory and antiangiogenic properties. Lenalidomide reduces the serum levels of TNF- $\alpha$  in ASD patients.<sup>55</sup> In addition, ASD patients have shown improved irritability, repetitive behavior and social interaction after lenalidomide administration.<sup>55</sup> Furthermore, lenalidomide inhibits the expression of inflammatory cytokines such as IL-1 $\beta$ , IL-6 and TNF- $\alpha$ , while it increases the expression of antiinflammatory cytokine IL-10 in Parkinson's disease model mice and  $\alpha$ -synuclein-stimulated activation of microglia *in vitro*.<sup>56</sup>

### 2.1.11 Spironolactone

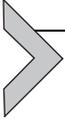
Spironolactone is a potassium-sparing diuretic that acts by antagonism of aldosterone in the distal renal tubules. Oral administration of spironolactone reduced irritability, hyperactivity and repetitive behavior in ASD patients.<sup>57</sup> Spironolactone also suppresses microglial secretion of inflammatory cytokines such as IL-1 $\beta$  and TNF- $\alpha$  induced by chronic compression of the dorsal root ganglion in rats.<sup>58</sup>

## 2.2 Cell-transplantation-based therapy

Cell-transplantation-based therapies may provide us with some clues regarding the future application of microglial transplantation as a therapeutic strategy for ASDs. Injection of bone marrow-derived mononuclear cell (BMMNC) intrathecally or subcutaneously<sup>68,69</sup> and embryonic stem (ES) cells intravenously<sup>70,71</sup> to ASD patients rescues ASD behaviors, including hyperactivity, repetitive behavior and impaired social communication. Additionally, intravenous injection of umbilical cord blood cells improved social communication in ASD patients.<sup>72</sup> Transplantation of cord blood mononuclear cells to veins in combination with umbilical cord-derived mesenchymal stem cells reduces repetitive behavior and improved sociability.<sup>73</sup>

It has also been suggested that BMMNC injection increases glucose metabolism at the prefrontal cortex and the hippocampus in ASD patients.<sup>68,69</sup> Glucose metabolism regulates activity-dependent synaptic vesicle transmission, suggesting the possible contribution of BMMNCs to the normalization of activity-dependent signal transmission.<sup>74</sup> This idea is supported by the transplantation of bone marrow mesenchymal stem cells into the cerebellum of a mouse model of Niemann-Pick disease: Purkinje cells increase spontaneous activity, promoting the formation of functional neural circuits after transplantation.<sup>75</sup> Recently, it has been reported that microglia-like cells can be generated from iPSCs, ES, and blood-derived monocytes.<sup>76-79</sup>

These microglia-like cells can be engrafted into the brain parenchyma, and they exhibit phagocytic properties.<sup>76</sup> Thus, it might be possible that microglia-like cells eliminate surplus synapses in the ASD brain. Moreover, microglia-like cells are suggested to be useful for drug screening.<sup>78,79</sup>



### 3. Synaptic deficits in ASDs

As mentioned above, immunomodulatory agents that suppress immune responses, such as luteolin and sulforaphane, are expected to be options for the treatment of ASDs; however, these strategies are symptomatic therapies. Furthermore, patients are usually treated with these agents chronically, which could cause multiple side effects. Another issue in ASD treatment is that it is difficult to determine the criteria for ASD severity because ASDs are spectrum disorders. In general, medication is prescribed if patients exhibit symptoms interfering with daily life, such as hyperactivity, aggression and self-injurious behavior. However, patients without those problems but with ASD-related problems, including deficits in social communication and their family, would also wish treatment for higher quality of life. These limits of medication led us to discover another therapeutic strategy.

In developing brains where synaptic pruning is robustly carried out, neural-activity-dependent signal transmissions are promoted, while they are suppressed after the critical period of developmental plasticity.<sup>80,81</sup> Deficits in developmental synapse elimination have been suggested to cause ASDs, but the mechanisms remain elusive. ASD patients usually suffer impaired processing of higher-order cognitive functions, but many reports imply abnormalities in primary sensory processing.<sup>82</sup> In sensory cortexes, including the visual and auditory cortices, neural activity induced by sensory stimuli causes neural circuits to form.<sup>83</sup> This circuit formation is regulated by modulation of synaptic maturation and function. Thus, it is possible that activity-dependent synaptic maturation and further circuit formation are not normally conducted in ASD brains.<sup>84</sup> Previous reports have demonstrated that microglia likely phagocytose less active synapses, indicating that microglia are able to decipher the strength of neuronal activity.<sup>85</sup> These findings have motivated us to examine whether microglia-dependent reorganization of synaptic connections could be a therapeutic strategy for ASDs; we hypothesize that microglia are unable to select synapses to phagocytose due to weak contrast between activities of each synapse in ASD brains.

Most animal models of ASDs are based on mutations of synapse-related genes, especially those that impair activity-dependent signal transmission (listed in [Table 2](#)).<sup>95</sup> For example, knockout of a member of the neuroligin

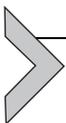
**Table 2** Animal models for ASDs.

Gene	Associated disease Function	Mutation Animal species	Behavioral abnormality	Neuroanatomical abnormality	Reference
NRX1	ASDs, schizophrenia, epilepsy, Pitt-Hopkins syndrome Presynaptic adhesion molecule	Nrxn1 $\alpha$ knockout C57BL6/ SV129 mice	Decreased social interaction	NA	86
SHANK	ASDs Postsynaptic scaffolding protein	Shank3 knockout C57 mice	Decreased social interaction, increased repetitive behavior	Decreased cortico-striatal synaptic transmission, striatal hypertrophy	87
		Shank2 knockout C57BL/6J mice	Decreased social interaction, impaired communication, enhanced anxiety	Impaired NMDAR function	88
FMR1	Fragile X syndrome mRNA trafficking, dendrite maturation, synaptic plasticity	Fmr1 knockout C57BL/6J mice	NA	Increased spines in hippocampal CA1, decreased microglia- dependent synaptic engulfment	15
		Fmr1 knockout C57BL/6 mice	NA	Dysregulation of glutamatergic signaling maturation	89
		Fmr1 knockout C57BL/6 mice	Impaired social interaction, increased repetitive behavior	Enhanced LTD and increased spines in hippocampal CA1	90

MECP2	Rett syndrome Transcriptional regulation	MeCP2 S421A knock-in C57BL/6 mice	Impaired social interaction	Increased dendritic complexity and increased mIPSC amplitude in visual cortex	91
PTEN	ASDs Inhibition of mTOR activation	Nse-cre; Pten <sup>loxP/loxP</sup> C57/BL6 mice	Decreased social interaction, decreased pre-pulse inhibition, decreased anxiety	Hippocampus hypertrophy, increased spines and ectopic dendrites in dentate gyrus	92
TSC1/2	Tuberous sclerosis Inhibition of mTOR activation	TSC1/2 hetero knockout C57BL/6J mice	Impaired context discrimination	Decreased LTD in hippocampal CA1	93
UBE3A	ASDs, Angelman syndrome	Ube3A knockout	NA	Increased Arc expression and decreased number of AMPAR at synapses in hippocampus	94

NA, not analyzed.

family, NRX1 $\alpha$ , leads to decreased social interaction,<sup>86</sup> which suggests the possible involvement of deficits in neural activity and synapse formation in ASDs because the structural binding of neurexin and neuroligin, which are located at the presynaptic and postsynaptic membranes, respectively, promotes synaptic maturation associated with neural activity.<sup>96,97</sup> SHANK2, a scaffold protein found in the postsynaptic densities of excitatory synapses, is crucial for normal long-term potentiation (LTP) and long-term depression (LTD).<sup>87</sup> SHANK2 knockout mice exhibit impaired social interaction, but the abnormal behavior is improved by restoring synaptic plasticity *via* NMDA receptor stimulation. Mutations in another SHANK-family protein, SHANK3, weaken excitatory neuronal transmission in corticostriatal synapses, dysfunctions of which are implicated in ASDs.<sup>88</sup> Risk genes for symptomatic ASDs, including fragile X syndrome and Rett syndrome, are also related to activity-dependent synaptic modulation. In *fmr1* knockout mice, a model of fragile X syndrome, activity-dependent synaptic maturation and pruning are impaired.<sup>89,90</sup> Recently, *fmr1* knockout has been revealed to reduce microglia-dependent synaptic pruning,<sup>15</sup> indicating the possible involvement of microglia in ASD-related symptoms. The Rett syndrome risk gene MECP2 is necessary for synaptic maturation.<sup>91</sup> Knockout of PTEN, an ASD risk factor gene, reduced synaptic pruning in the hippocampus.<sup>92</sup> Mutations of the tuberous sclerosis risk gene TSC1/2, which encodes a downstream protein of PTEN, hinder activity-dependent gene translation and synaptic plasticity.<sup>93</sup> In an Angelman model, mice with mutations in UBE3A, which is also an ASD risk gene, exhibit reduced activity-dependent gene expression and excitatory neural transmission.<sup>94</sup>



#### **4. Physical exercise, possibly a potent therapeutic strategy for ASDs**

Mild therapeutic strategies for ASDs, as opposed to drug or cell-transplantation therapies, are ideal. Recently, exercise has attracted attention as a potential therapeutic strategy for abnormal behaviors in neurodevelopmental disorders, including ASDs, but the cellular and molecular mechanisms by which exercise ameliorates the symptoms remain unrevealed.<sup>98–100</sup> In the following paragraphs, we discuss the possibility that physical exercise is a viable treatment to induce synaptic pruning by microglia, resulting in improved ASD-related behaviors.

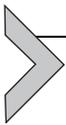
Previous studies have reported that exercise may help improve cognitive performance in humans and rodents, probably through enhancing the

production of neurotrophic factors and increasing hippocampal neurogenesis.<sup>101–104</sup> The hippocampus and prefrontal cortex regulate cognition and social behavior, deficits of which are the main symptoms of ASD, highlighting these regions as possible target brain regions for ASD treatment.<sup>105</sup> Consistent with this possibility, exercise increases neural activity in multiple regions including the hippocampus and prefrontal cortex in humans and rodents.<sup>106,107</sup> These regions show increased expression of brain-derived neurotrophic factor (BDNF) and insulin-like growth factor-1 (IGF-1) in response to exercise.<sup>108,109</sup> BDNF is secreted depending on neural activity, promoting synaptic maturation.<sup>110</sup> IGF-1 also stabilizes synapses by increasing the expression of synapse-related molecules such as Synapsin I and PSD95.<sup>111,112</sup> These molecules may accelerate activity-dependent synaptic maturation after exercise.

Another major effect of exercise is the enhancement of adult neurogenesis in the hippocampus.<sup>104</sup> Adult-born hippocampal granule cells exhibit higher excitability than preexisting mature granule cells, which may generate synaptic competition between newborn and existing cells.<sup>113</sup> Thus, it is possible that microglia detect differences in neural activity therein. It was recently revealed that in MIA-induced ASD model mice, hippocampal neurogenesis is inhibited during both the immediate postnatal period and adulthood, leading to the impaired maturation of newborn granule cells.<sup>114</sup> Thus, inadequate neurogenesis may underlie ASD development. The activity of neural precursor cells is also enhanced by exercise, and this process is modulated by the CX3CL1-CX3CR1 axis, one of the important signaling pathways regulating microglia-neuron interaction, suggesting an active role of microglia in the exercise-induced activity of neural precursors.<sup>115</sup>

In order to generate contrast in synaptic activity, which is necessary for microglia-dependent synaptic pruning, it will be also useful to focus on inhibitory neurotransmission, as GABAergic signaling has been shown to regulate synaptic pruning in the cerebellum.<sup>116</sup> In addition, impaired inhibitory neurotransmission has been reported in some animal models of ASDs.<sup>117–119</sup> Furthermore, ASD patients express reduced levels of glutamic acid decarboxylase (GAD) and GABA receptor subunits, which implies a causal relationship between impaired inhibitory neural transmission and ASDs.<sup>120–122</sup> Exercise increases the numbers of interneurons and inhibitory synapses, enhancing the secretion of GABA.<sup>123,124</sup> Therefore, exercise is expected to induce synaptic competition by modulating both excitatory and inhibitory neural transmission.

Neural activity promotes the dynamics of microglial processes *in vivo*.<sup>125</sup> Therefore, elevation of neural activity by exercise might induce microglial contact with synapses, resulting in the elimination of less active synapses by microglia. It has been reported that exercise decreases the expression of MHC-II and CD68 in microglia, both of which are regarded as markers of activated microglia.<sup>126,127</sup> These results indicate that exercise induces synaptic pruning without overactivating microglia. We are now testing the hypothesis that exercise induces microglia-dependent synaptic pruning through synaptic competition. Our study will link exercise to microglia-dependent changes in the status of the brain.



## 5. Concluding remarks

We discussed the involvement of microglia in ASDs and the possibility of microglia as the therapeutic targets for ASDs. As introduced in the first section, several researches have suggested that microglia are likely involved in the pathogenesis and development of ASDs. In the second section, we introduced immunomodulatory agents that could attenuate ASD-related behaviors probably *via* regulation of microglial functions. Some of them are under clinical trial, but the side effects should be carefully addressed before the actual clinical use. We also mentioned the cell-transplantation-based therapy, but the method itself is invasive and could result in undesired activation of innate microglia. In the third and fourth sections, we further focused on synaptic remodeling by microglia in the ASD brain. Specifically, we mentioned the possibility that exercise cures ASD behaviors *via* inducing synaptic pruning by microglia. Though it should be noted that some ASD patients have difficulties in exercise or may feel stress by compulsive exercise, exercise, if it is effective, is useful in that it can be basically a noninvasive treatment and the exercise intensity can be changed according to the severity of ASD symptoms. A few clinical studies have shown that various types of exercise actually ameliorate ASD-related behaviors. We are currently examining the cellular and molecular mechanisms underlying exercise-induced modulation of ASD behaviors.

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