expression, indicating onset of apoptosis in zebrafish brain.[48] Dusp1 can mediate MAPK signaling and inflammation.[49] The downregulation of *Dusp1* in the Ag-NPS treatment group activates the MAPK signaling pathway, which induces inflammation and apoptosis. KEGG pathway analysis also showed that the MAPK signaling pathway was affected in astrocytes after exposure to Ag-NPS, but not after exposure to released Ag ions. In general, compared with released Ag ions, Ag-NPS induced more toxicity by generating ROS and significantly decreasing the expression of neuroprotective genes against ROS in astrocytes, weakening astrocytes' ROS protective ability, and subsequently inducing inflammation and apoptosis. Taken together, our results were consistent with previous research,[50] which showed that ROS generation, inflammatory reactions, and apoptosis are the primary mechanisms of Ag-NP induced toxicity.[19,48]

Previous research has demonstrated that astrocytes play an important role in providing polyunsaturated fatty acids to the BBB and brain.[51] Polyunsaturated fatty acids are ROS targets in the brain due to their high metabolic rate and their rich composition in the brain,[52] which make astrocytes more sensitive to Ag-NP-induced ROS and more susceptible to losing their defense ability. In the present study, antioxidant defense was inhibited in astrocytes following Ag-NPS exposure. Meanwhile, Ag-NPS also induced inflammation and apoptosis through the MAPK pathway, Bcl-2 expression, or mTOR activity in astrocytes. The results raise concerns regarding Ag-NP toxicity in astrocytes. More studies are needed to understand how the toxicity effects induced by Ag-NPs in astrocytes influence neuron functions.

In the present study, 3 learning/memory/cognition processes, 11 developmental processes, 31 metabolic processes, and 24 biosynthetic processes were affected in astrocytes after Ag-NPS exposure (Table S3). Our results are consistent with previous studies that demonstrated that Ag-NPs can reduce learning and cognition of rats[53] and can produce developmental neurotoxicity and behavioral effects.[54] Furthermore, the metabolic relationship between astrocytes and neurons is critical for energy metabolism and the synthesis of neurotransmitters.[55] We speculate that induction of a large amount of changes in metabolic processes of astrocytes by Ag-NPS could significantly influence neuronal functions. Astrocytes also release factors that sustain neuronal function and viability. Astrocytes synthesize and secrete a wide range of neurotrophic and growth factors, cytokines, extracellular matrix proteins, proteoglycans, and cholesterol, which are involved in neuronal survival, proliferation, differentiation, and synaptogenesis.[56] Ag-NPS exposure significantly inhibited biosynthetic processes in

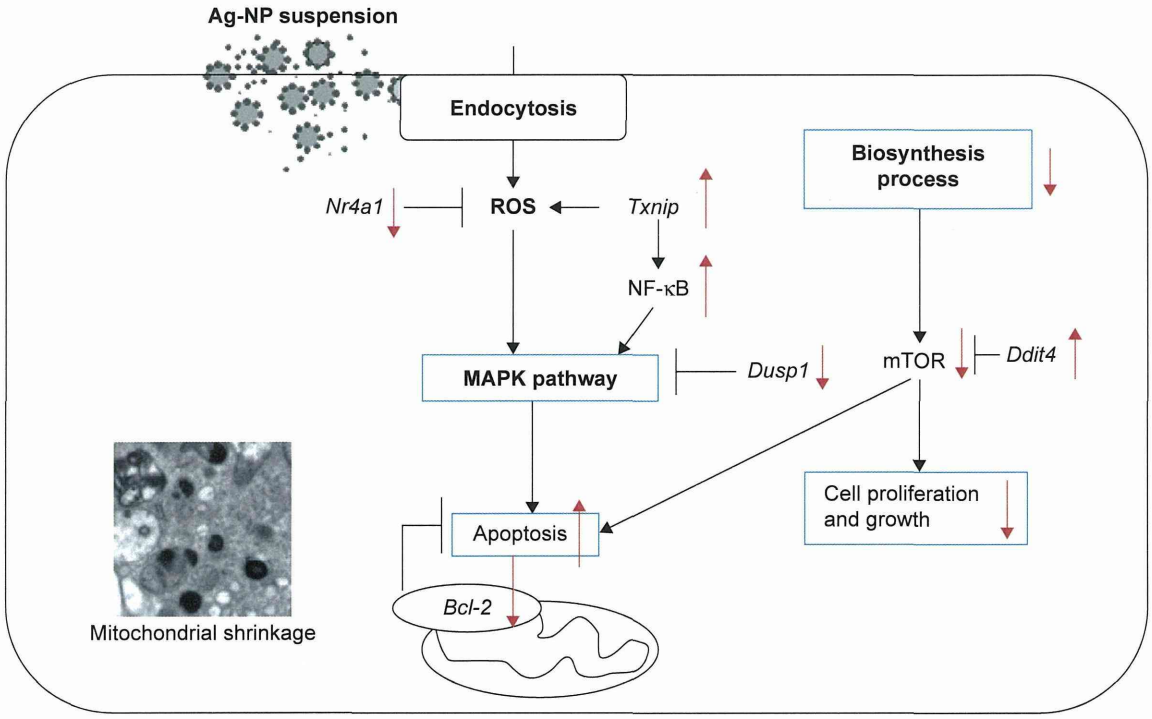

Figure 8 Mechanisms of neurotoxicity induced by Ag-NPS in astrocytes based on the data from the present investigation.
Abbreviations: Ag-NPS, silver nanoparticle suspension; Bcl-2, B-cell lymphoma-2; ROS, reactive oxygen species; Txnip, thioredoxin interacting protein.

astrocytes and may decrease the secretion of important nutrients and signal factors. Further studies are needed to investigate how the toxicity effects induced by Ag-NPs in astrocytes influence neuronal survival, proliferation, and differentiation to better understand the mechanisms of Ag-NP-induced neurotoxicity.

Conclusion

We established a BBB model using microvascular endothelial cells, pericytes, and astrocytes, which enhanced levels of TJ proteins (claudin-5 and ZO-1), with TEER >200 $\Omega \cdot cm^2$ on Day 5. After 10 μg/mL Ag-NPS exposure for 24 hours, the BBB permeability was significantly increased and ZO-1 expression was significantly decreased compared to control. Severe mitochondrial shrinkage, ER expansion, and nuclear atypia were observed in astrocytes. Global gene expression analysis showed 23 significantly changed genes that were associated with several biological processes. As illustrated in Figure 8, Ag-NPS significantly increased *Txnip* expression, which modulated antioxidant defense. On the other hand, Ag-NPS significantly decreased *Nr4a1* and *Dusp1*, which protect cells from oxidative stress, inflammation, and apoptosis. Ag-NPS induced toxicity by decreasing antioxidant defense in astrocytes. Meanwhile, Ag-NPS also induced ROS, inflammation, and apoptosis through modulation of the MAPK pathway or Bcl-2 expression or mTOR activity in astrocytes. Ag-NPS also inhibited eleven developmental processes and caused learning and cognition reduction. Furthermore, Ag-NPS significantly suppressed 31 metabolic and 24 biosynthesis processes in astrocytes, which may influence the main function of astrocytes in the CNS and increase the risk of Ag-NP-induced neurotoxicity.

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Disclosure

The authors report no conflicts of interest in this work.

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