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Cellular interplay in brain organoids: Connecting cell-autonomous and non-cell-autonomous mechanisms in neurodevelopmental disease

Giovanna Berto[1,3,b], M. Veronica Pravata[1,b] and Silvia Cappello[1,2,a]

The field of brain organoids has experienced a period of rapid and transformative growth, enabling researchers to investigate complex human biological mechanisms that were previously deemed intractable. This review provides an overview of the current landscape of brain organoids, with a particular focus on their relevance in the context of neurodevelopmental disorders. It also emphasizes the crucial role these models play in elucidating both cell-autonomous and non-cell-autonomous mechanisms. We describe how these two mechanisms, often considered to be independent, are intricately interconnected. In conclusion, this review aims to highlight how the utilization of brain organoids has considerably advanced our comprehension of neurodevelopmental disorders, while also delineating prospective avenues for investigating these complex conditions.

Addresses
[1] Division of Physiological Genomics, Biomedical Center (BMC), Faculty of Medicine, Ludwig Maximilian University (LMU), Munich, Germany
[2] Max Planck Institute of Psychiatry, Munich, Germany
[3] International Max Planck Research School for Translational Psychiatry, Munich, Germany

Corresponding author: Cappello, Silvia (silvia.cappello@bmc.med.lmu.de)
[a] Lead Contact.
[b] These authors contributed equally to this work.

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Introduction

The notion of cell autonomy is central during development, when cells undergo a series of intricated processes that involve both cell-autonomous and non-autonomous mechanisms. The former refers to events where genetic mutations and epigenetic changes directly affect a cell's behavior, independent of surrounding signals. In contrast, non-cell-autonomous mechanisms involve the influence of neighboring cells or extracellular signals on a cell's function. Understanding the balance between cell-autonomous and non-autonomous processes is critical for many aspects of developmental biology. Therefore, the interplay between these mechanisms is crucial to unravel the complex etiology of neurodevelopmental disorders (NDDs), such as autism spectrum disorder (ASD) [1,2] (Box 1), and cortical malformations (CMs) [3].

Box 1: CHOOSE-seq - a scalable approach for studying multifactorial neurodevelopmental disorders

Traditional approaches to studying NDDs in organoid models often focus on single-gene perturbations, limiting insight into how multiple disease-associated genes interact within complex cellular ecosystems. A recent breakthrough, CHOOSE-seq (CRISPR-human organoid-single-cell RNA-sequencing), developed by Li et al., provides a scalable solution by enabling the simultaneous perturbation of multiple ASD-related genes within the same organoid system [34]. This approach combines CRISPR gene editing with single-cell RNA sequencing, allowing researchers to systematically assess how different genetic variants affect cell identity, fate and transcriptomic profiles. Applying CHOOSE-seq to ASD, the study found that specific progenitor and neuronal subpopulations, including dorsal intermediate progenitors, ventral progenitors and upper layer excitatory neurons, are particularly sensitive to disruptions in high-risk ASD genes. Importantly, their findings suggest that ASD pathophysiology involves both convergent and divergent mechanisms, emphasizing shared pathways between multiple genes as well as gene-specific effects.

Limitations and considerations: While CHOOSE-seq represents a major advance in high-throughput screening of disease-associated genes, its mosaic nature poses a significant challenge for the study of non-autonomous cell effects. Because genetically modified cells are interspersed in a mixed population of wild-type and mutant cells, it remains difficult to track how mutations affect neighboring cells or the extracellular environment. Future adaptations of this technology - such as engineered microenvironments or spatially controlled perturbations - may help to overcome this limitation.

Brain organoids have emerged as transformative tools in disease and basic research, offering an unprecedented

opportunity to study complex events that underlie neurodevelopmental disorders and other conditions [4]. Brain organoids provide an ideal platform for modelling human-specific phenotypes, enabling the dissection of cell-autonomous and non-cell-autonomous mechanisms. This has been possible thanks to recent advancements in the field, which include assembloids - connected organoids from distinct brain regions - and neuro-immune organoids - organoids with incorporated microglia - enabling researchers to study non-cell-autonomous effects in region-specific and immune-related aspects of brain disorders [5–7]. Additionally, models such as mosaic cerebral organoids and chimeroids are gaining attention for their ability to dissect such interactions. Mosaic organoids incorporate cells with distinct genetic backgrounds and allow direct comparison of cell-autonomous effects (e.g., mutation-specific cellular behavior) and non-cell-autonomous impacts (e.g., altered secretion of extracellular vesicles from mutant cells affecting neighboring wild-type cells) [8–11]. Meanwhile, brain chimeroids, composed of human induced pluripotent stem cell (iPSC)-derived neural progenitor cells (NPCs) with multiple genetic backgrounds, have been employed to investigate responses to neurotoxic stimuli, revealing how genetic diversity shapes susceptibility to neurological disorders [12]. Given the complexity of these models, rigorous experimental design is essential to ensure accurate interpretation of results. For best practices in organoid handling, we refer the reader to recent guidelines in the field [13].

Through this review, we aim to present the state of the art in the field, highlighting the latest key achievements and illustrating how brain organoids have advanced our understanding of both cell-autonomous and non-cell-autonomous mechanisms. Importantly, we highlight how closely interconnected, yet essential, these mechanisms are in the context of brain development.

Cell-autonomous mechanisms in neurodevelopmental disorders

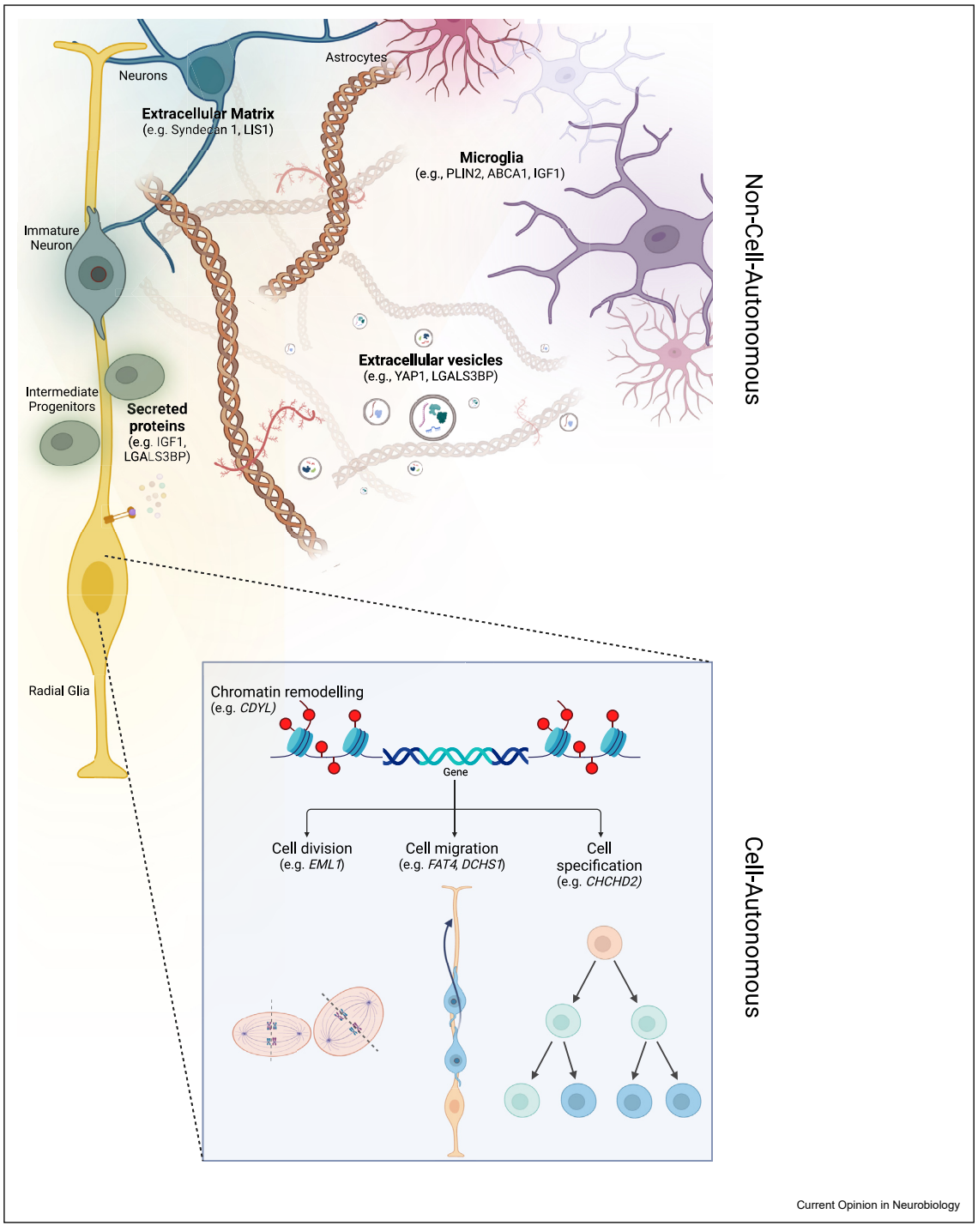
Neurodevelopmental disorders (NDDs) result from the interaction of multiple genetic and environmental factors, often involving both cell-autonomous and non-cell-autonomous mechanisms. While some genes exert predominantly cell-intrinsic effects, others influence the microenvironment, intercellular signaling or extracellular matrix (ECM) composition, thereby contributing to non-cell-autonomous disease mechanisms. A significant subset of NDDs are cortical malformations (CMs) - a heterogeneous group of developmental disorders characterized by abnormalities of cortical structure such as subcortical band heterotopia, lissencephaly, periventricular heterotopia and polymicrogyria [3,14]. These disorders often result from abnormalities in neuronal proliferation, migration or differentiation and are frequently associated with epilepsy, intellectual disability and motor dysfunction. The genetic basis of CMs involves both cell-autonomous defects (e.g. impaired progenitor behavior, disrupted cytoskeletal dynamics) and non-cell-autonomous factors (e.g. altered ECM composition, defective intercellular signaling).

Genes with predominantly cell-autonomous effects in NDDs

Several genes associated with CMs are strongly linked to cell-autonomous defects in progenitor proliferation, differentiation, or neuronal migration, with little evidence for cell-extrinsic contributions. Mutations in *FAT4* and *DCHS1*, which are associated with periventricular heterotopia, primarily affect intrinsic neuronal morphology and synaptic function [15] (Figure 1). Transcriptomic and proteomic analyses of *FAT4* and *DCHS1* mutant brain organoids identified changes in gene ontology terms related to neuronal morphology and synaptic activity. Electrophysiological recordings revealed that these neurons have increased expression of somatic voltage-gated sodium channels, contributing to hyperactivity. In addition, morphological reconstructions and immunostaining revealed increased structural complexity in periventricular heterotopia neurons. Importantly, the expression of wild-type DCHS1 rescued the morphological phenotype, supporting its role as a cell-autonomous regulator of neuronal structure [15].

Beyond genes associated with cortical malformations, other neurodevelopmental genes with cell-autonomous roles have also been implicated in disease. One such example is *CHCHD2*, a gene primarily associated with mitochondrial function and stress responses [16–18] but increasingly linked to neurodevelopmental disorders, including Huntington's disease (HD) [19]. While *CHCHD2* is primarily recognized for its role in mitochondrial dynamics, recent studies suggest a broader function in neurodevelopmental processes, particularly in axon guidance, mTOR signaling, and progenitor cell behavior. This places *CHCHD2* in a growing category of genes with cell-autonomous effects that may contribute to NDDs. Cerebral organoids harboring mutant huntingtin exhibit disorganized and improperly specified NPCs, accompanied by immature ventricular zones. Moreover, neuronal cultures from HD patients show disorganized NPCs, immature ventricular zones and impaired neurite outgrowth, which are associated with *CHCHD2* downregulation [20]. *CHCHD2* has also been identified as a key player in axon guidance, Hippo signaling and mTOR pathways in HD models. Notably, *CHCHD2* expression is reduced in iPSC-derived neurons from patients with the CM genes *LIS1* or *TUBA1A* mutations, suggesting a broader role in

Figure 1



Overview of reviewed non-cell-autonomous and cell-autonomous mechanisms. Illustration of non-cell-autonomous and cell-autonomous mechanisms. Non-cell-autonomous mechanisms encompass astrocytes and microglia that influence nearby neurons through interactions with the ECM, EVs, and secreted proteins. Cell-autonomous mechanisms comprise intrinsic mechanisms, such as cell division, cell migration, and cell specification. Created with BioRender.com.

neurodevelopment [21]. Given its function in regulating intracellular processes, *CHCHD2* appears to act predominantly through cell-autonomous mechanisms.

Genes with both cell-autonomous and non-cell-autonomous effects

Some neurodevelopmental genes act primarily in a cell-autonomous manner but also exert secondary non-cell-autonomous effects by modifying the extracellular environment, intercellular signaling or other microenvironmental factors. This interplay suggests a crosstalk between cell intrinsic and extrinsic mechanisms.

One striking example of this crosstalk is *EML1*, a gene associated with subcortical heterotopia, which illustrates how intrinsic mutations can affect the ECM. Jabali et al. showed that *EML1*-deficient brain organoids exhibit ectopic neuronal rosettes, disrupted progenitor behavior and excessive ECM deposition [22]. The increased ECM production is associated with YAP1-mediated progenitor expansion, illustrating how a cell-autonomous defect can have non-cell-autonomous consequences for neighboring cells. Importantly, brain organoids provide a powerful model to study these mechanisms, particularly as modelling heterotopia-associated genes in mice has proven challenging. For example, *Eml1* mouse models exhibit abnormal cell proliferation, perturbations in apical radial glial (RG) behavior, and defects in primary ciliary structure [23,24]. While these models develop neuronal heterotopia, they fail to recapitulate key human-specific phenotypes such as the polymicrogyria-like cortex or megalencephaly observed in patients [25]. The ability of organoids to capture species-specific developmental processes makes them an invaluable tool for studying neurodevelopmental disorders that are not fully represented in traditional animal models.

Similarly, *CDYL*, an epigenetic regulator of histone modification, exerts both intrinsic and extrinsic effects. Cell-autonomously, it plays a key role in neuronal excitability, synaptic plasticity and early neural development [26,27], in part by facilitating the establishment and spreading of H3K27me3, a histone modification associated with gene silencing. CDYL deficiency in ASD patient-derived brain organoids has been associated with disruptions in GABAergic neuron generation mediated by modulation of the WNT and SHH signaling pathways [28]. Beyond its intrinsic role, CDYL also exerts non-cell-autonomous effects by influencing the extracellular environment. Notably, it regulates ECM-related genes such as *NNAT*, suggesting a role in influencing the local microenvironment, providing a clear example of crosstalk between cell-autonomous and non-cell-autonomous effects. Moreover, *NNAT* is not highly expressed in mice, suggesting potential evolutionary differences in neurogenesis regulation. This highlights the value of brain organoids in modeling species-specific mechanisms in neurodevelopmental disorders.

Beyond *CDYL*, another ASD-associated gene, *SYNGAP1*, also exhibits both cell-autonomous and non-cell-autonomous roles. Traditionally recognized as a postsynaptic scaffolding protein, *SYNGAP1* plays a critical role in synaptic activity, where it is essential for assembling the core scaffold machinery of the post-synaptic density in excitatory synapses [29,30]. However, an emerging body of evidence suggests that some ASD-related genes also function earlier in neurodevelopment. Recent findings by Birtele et al. highlight an unexpected role for *SYNGAP1* in early neurogenesis, where it is highly expressed in RG cells and regulates cytoskeletal remodeling of subcellular components [31]. Given that RG cells secrete signaling molecules and patterning cues, SYNGAP1 mutations may not only affect intrinsic cellular processes but also influence the fate and behavior of surrounding progenitors. This underscores the importance of brain organoids in elucidating early developmental effects of ASD genes, revealing how mutations in genes traditionally associated with synaptic function may also have non-cell-autonomous effects during cortical development.

Epigenetic mechanisms are also involved in this interplay [32]. Ditzer et al. investigated the epigenetic landscape of human fetal brain tissue and cortical organoids using Epi-CyTOF, an innovative mass cytometry technique that enables the profiling of histone modifications at the single-cell level [33]. Their analysis identified H3K27me3 as a major regulator of NPCs fate decisions, with this repressive histone modification being mediated by Polycomb Repressive Complex 2 (PRC2). Functionally, inhibition of PRC2 in cortical organoids induced a shift in NPC proliferation toward differentiation, demonstrating its role in controlling cell-autonomous neurogenic gene expression. However, PRC2 also non-cell-autonomously regulates NPC fate by influencing ECM composition, highlighting a crosstalk between intrinsic and extrinsic mechanisms. Specifically, PRC2 target genes regulate neuronal differentiation, netrin signaling, and ECM components such as syndecan 1, illustrating how epigenetic regulation can simultaneously shape both cell-intrinsic and microenvironmental properties in the developing brain (Figure 1).

Non-cell-autonomous effects in neurodevelopmental disorders

Non-cell autonomous cues typically encompass secreted factors, such as cytokines, growth factors, and neurotransmitters, as well as extracellular vesicles (EVs), which mediate intercellular communication. Additionally, these cues include external environmental influences, such as the ECM, which provides structural

and biochemical support to cells, and direct or indirect interactions between different cell types, including neuron-glia crosstalk, immune cell signaling, and vascular-endothelial interactions. Together, these mechanisms play a crucial role in shaping cellular behavior in both physiological and pathological contexts (Figure 1).

Non-cell-autonomous effects mediated by extracellular signals

EVs are small particles released by cells that transfer proteins, nucleic acids, and lipids to other cells, influencing their behavior [35]. In recent years, a great deal of attention has been devoted to understanding how the release and content of EVs affect neighboring cells. Most of the studies have been performed in the field of cancer biology and neurodegenerative diseases [36–38], and little is known about the influential effect of EVs on intercellular crosstalk during neurodevelopment. Forero and colleagues have made significant strides in addressing this gap [10]. Their findings reveal that EVs possess protein content and dynamics specific to each cell type, which evolve over time - likely as a result of increased cell heterogeneity. Interestingly, EVs were shown to transport important regulatory molecules across cells, such as the transcription factor YAP1, with different effects depending on the recipient cell type. This study suggests a critical role for EVs in trafficking, facilitating intercellular communication and regulating processes like neurogenesis.

In addition, new insights into EVs during neurodevelopment come from Pipicelli et al. [8]. They investigated how EVs regulate neuronal specification and migration using ventral cerebral organoids (vCOs) and dorsoventral cerebral assembloids (dvCAs). They investigated how a point mutation in the secreted extracellular matrix gene *LGALS3BP*, previously associated with NDDs, affects these processes. It was shown that mutant vCOs and dvCAs display dorsal identity and migratory defects. Interestingly, EVs derived from mutant cerebral organoids show altered protein composition, specifically related to neuronal migration, cell fate and ECM composition. They identified proteins whose genes are associated with CMs, ASD and epilepsy. These alterations led to differences in dorsoventral patterning, suggesting that EVs may play a key role in regulating neuronal identity. In addition, treatment of dissociated control vCOs and NPCs with mutant EVs inhibited ventralization and altered the transcriptomic profile of NPCs, activating the WNT and NOTCH pathways, which are critical for cell fate determination and regionalization [39]. Treatment with control EVs restored ventral gene expression in mutant cells, highlighting that LGALS3BP regulates interneuron specification through EV-mediated communication (Figure 1).

The ECM is a non-cellular scaffolding component that supports three-dimensional cell growth and has recently been shown to play a key role in neurodevelopment in a non-cell-autonomous manner [40,41] (Figure 1). The composition and mechanical properties of the ECM have been shown to have a critical effect on many cell functions, including cell anchorage, signaling, cell survival, and disease progression [42]. Recently, some new advances have been made in studying and understanding how the stiffness of the brain, and therefore the composition of the ECM, affects brain development. Zur and colleagues have focused on understanding how tissue mechanics affects neuronal development and disease states [43]. They examined how mutations in the *LIS1* gene, which is associated with lissencephaly (a brain malformation characterized by the absence of cortical folds), affect ECM organization [44,45]. The researchers found that brain organoids carrying *LIS1* mutations are significantly stiffer than control organoids at several developmental stages. This stiffness is attributed to abnormal ECM composition as well as increased water content within the tissue. Mass spectrometry revealed that ECM-related proteins, particularly collagens, were enriched in the mutant organoids, indicating a mutation-induced alteration in ECM secretion and remodeling. Application of metalloproteinase MMP9, an enzyme that degrades ECM components, reduced stiffness in the mutant organoids, suggesting that ECM-related changes may be reversible. These findings provide insight into LIS1 as a key regulator of the ECM and highlight the importance of the ECM in shaping tissue, influencing cellular behavior and contributing to brain development.

Non-cell-autonomous effects mediated by cell-cell interactions

Intercellular communication is thought to be a critical contributor to brain development. However, in standard brain organoid models, key non-neuronal cell types such as microglia, astrocytes, and endothelial cells are either absent or underrepresented, limiting their ability to fully recapitulate the complexity of the developing brain. Lately, significant efforts have been made to implement these cells in the organoid field to understand how neuronal and non-neuronal cell types interact to shape neural development (Figure 1).

Among these, glia-enriched cortical organoids have been recently generated by Wang and colleagues by inducing an early gliogenic switch in forebrain organoids [46]. The glia-enriched organoids have been intracerebrally transplanted in immunodeficient mice to better mimic a brain environment, enabling to study the function and diversity of human astrocytes. Similarly, in the last years, the integration of human endothelial cells and pericytes led to advancements in vascularized brain organoids, allowing the investigation of neurovascular interactions [47].

While various cell types have also been integrated into brain organoids to explore intercellular communication, microglia-mediated crosstalk has been increasingly recognized as a key factor in NDDs. To study the interaction of human microglia (hMGs) with their human neuronal environment, Schafer and colleagues generated an immunocompetent human brain organoid (iHBO) model co-cultured with iPSC-derived erythromyeloid progenitors (EMPs) [48]. By xenotransplanting iHBOs into mice, Schafer studied hMGs in a vascularized, human brain-like environment *in vivo*. The study shows that hMGs populate the organoid, express specific microglial markers (e.g., *P2RY12*, *TMEM119*, *SALL1*) [49], and exhibit morphological characteristics of a quiescent, surveillance state. The model supports the study of hMG behavior under both physiological and pathological conditions. It has proven to be particularly valuable for studying microglial involvement in ASD with macrocephaly. Microglia from ASD-derived organoids exhibited morphological changes (e.g., increased soma size and thicker processes) consistent with a primed and reactive state. Importantly, these changes were driven by the ASD brain environment rather than intrinsic microglial factors, as demonstrated by experiments with control microglia in ASD environments.

Further advances in microglia crosstalk were made by Park et al. They co-cultured brain organoids with iPS cell-derived primitive macrophages (iMac) that differentiate into microglia-like cells (iMicro) [50]. The addition of microglia to the brain organoid promoted neuronal differentiation, limited NPC proliferation and promoted axogenesis. The researchers highlight the involvement of microglia in cholesterol transfer, a critical process for NPC metabolism and differentiation. iMicro highly expresses *ABCA1* and *PLIN2*, suggesting a role in lipid metabolism that promotes NPC differentiation into neurons (Figure 1). These findings indicate that microglia have a significant impact on forebrain development by modulating neuron and axon growth.

Finally, Yu and colleagues shed light on the role of human microglia in the development of inhibitory GABAergic neurons, which primarily arise from the medial ganglionic eminence (MGE) [51]. Alterations in the number and function of interneurons are known to be associated with several neurological disorders, including ASD, but little is known about their development. To investigate the role of microglia in GABAergic neurons during early development, the researchers established a human MGE neuroimmune organoid (MGEO) model by transplanting human embryonic stem cell (ESC)-derived microglia (iMG) into hPSC-derived MGE organoids. They found that microglia secrete insulin-like growth factor 1 (IGF1), which enhances the proliferation of MGE progenitors and thereby regulates the production of interneurons (Figure 1). These findings suggest a key role for microglia-IGF1 signaling in the formation of inhibitory neural circuits during brain development.

Final remarks

In summary, the study of both cell-autonomous and non-cell-autonomous mechanisms in brain organoids provides valuable insights into the complex nature of NDDs. These mechanisms interact synergistically in multiple ways. One form of interaction occurs when a cell-intrinsic genetic alteration modifies intracellular pathways that in turn affect neighboring cells or the extracellular environment, such as mutations in cytoskeletal genes leading to excessive ECM secretion, which alters signaling and cell behavior in the surrounding microenvironment. Another mode of interaction involves a cell-autonomous defect being modified, exacerbated, or even rescued by non-cell-autonomous factors, for example, paracrine signaling from wild-type neighboring cells mitigating differentiation defects in mutant progenitors. Brain organoid models have evolved to recapitulate key features of brain structure and function, as they enable different genetic backgrounds and diverse cell types to coexist within a single system, providing a unique tool to dissect the complex interplay between intrinsic mutations and external signals in shaping human brain development and disease progression. However, it is important to recognize the limitations of these models - such as variability due to iPSC reprogramming and limited neuronal connectivity and maturation, particularly when cultured *in vitro* rather than transplanted [52]. Nevertheless, these models hold great promise for unravelling the multifactorial nature of NDDs, providing avenues for targeted therapeutic intervention and advancing precision medicine in neurodevelopmental and neurodegenerative research [53].

Author contributions

GB and MVP wrote, edited, and illustrated the manuscript. SC reviewed and edited the manuscript.

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Declaration of competing interest

The authors declare no conflict of interest.

Data availability

No data was used for the research described in the article.

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- •• of outstanding interest

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