JOURNAL OF INTEGRATED OMICS
A METHODOLOGICAL JOURNAL
HTTP://WWW.JIOMICS.COM

PERSPECTIVE ARTICLE | DOI: 10.5584/jiomics.v16i1.253

Integrating Omics into Endocannabinoid Research: a Paradigm Shift Toward a System-Level Understanding of a Dynamic Biological Network

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Available Online: Septemper 2026

ABSTRACT

Originally identified as the molecular machinery responsible for mediating the effects of cannabis-derived compounds, the endocannabinoid system (ECS) is now recognized as having a role in the regulation of many biological processes. Its contribution to physiological homeostasis relies on the coordinated activity of cannabinoid receptors, endogenous lipid mediators, metabolic enzymes and interconnected signaling pathways. Despite substantial advances in ECS research, several aspects of its regulation and functional relevance remain not completely understood. Many studies have traditionally focused on individual molecular components, involving either cannabinoid receptors or specific enzymes responsible for endocannabinoid metabolism. Although these approaches have provided essential mechanistic knowledge, they may not fully represent the dynamic complexity of the ECS, whose activity varies according to genetic background, metabolic state, environmental factors, cellular identity, tissue context, and disease progression. The integration of omics may provide a broader framework for investigating this complex system. Genomics, transcriptomics, proteomics, lipidomics, and metabolomics offer complementary molecular perspectives that allow the ECS to be examined beyond individual pathways and within a systems biology context. These approaches may contribute to the identification of regulatory mechanisms, molecular signatures associated with diseases, potential biomarkers, and factors influencing therapeutic response. The convergence of endocannabinoid biology and multi-omics technologies may support a more comprehensive representation of the ECS as a multidimensional and context-dependent biological network. This perspective paper discusses the current contribution of omics to the study of ECS physiology and dysfunction, together with their potential applications and the methodological and translational challenges that still need to be addressed.

Keywords: Endocannabinoid system; Endocannabinoidome; Cannabinoid signaling; Omics; System biology.

1. From Cannabinoid Receptors to a Complex Regulatory Network

The discovery of the endocannabinoid system (ECS) has broadened our understanding of cellular communication and physiological regulation. For decades, cannabinoids were investigated primarily for their psychoactive and pharmacological effects. The subsequent identification of cannabinoid receptors and endogenous ligands revealed the existence of an intrinsic signaling system that extends far beyond the pharmacological effects of cannabis-derived molecules. The ECS is now recognized as a fundamental regulator of biological homeostasis, contributing to maintaining the equilibrium between different physiological processes, including synaptic transmission, immune and inflammatory responses, appetite regulation, energy metabolism and stress adaptation [1,2]. The core components of the ECS include cannabinoid receptors, endogenous cannabinoid ligands, and the enzymes involved in their biosynthesis and degradation (**Figure 1**) [1,2].

Cannabinoid receptors CB1 and CB2 are the most extensively characterized elements of this system [3]. CB1 is among the most abundant G protein-coupled receptors in the central nervous system (CNS), where it regulates neurotransmitter release and contributes to synaptic plasticity, learning, memory, and emotional behavior [2,3].

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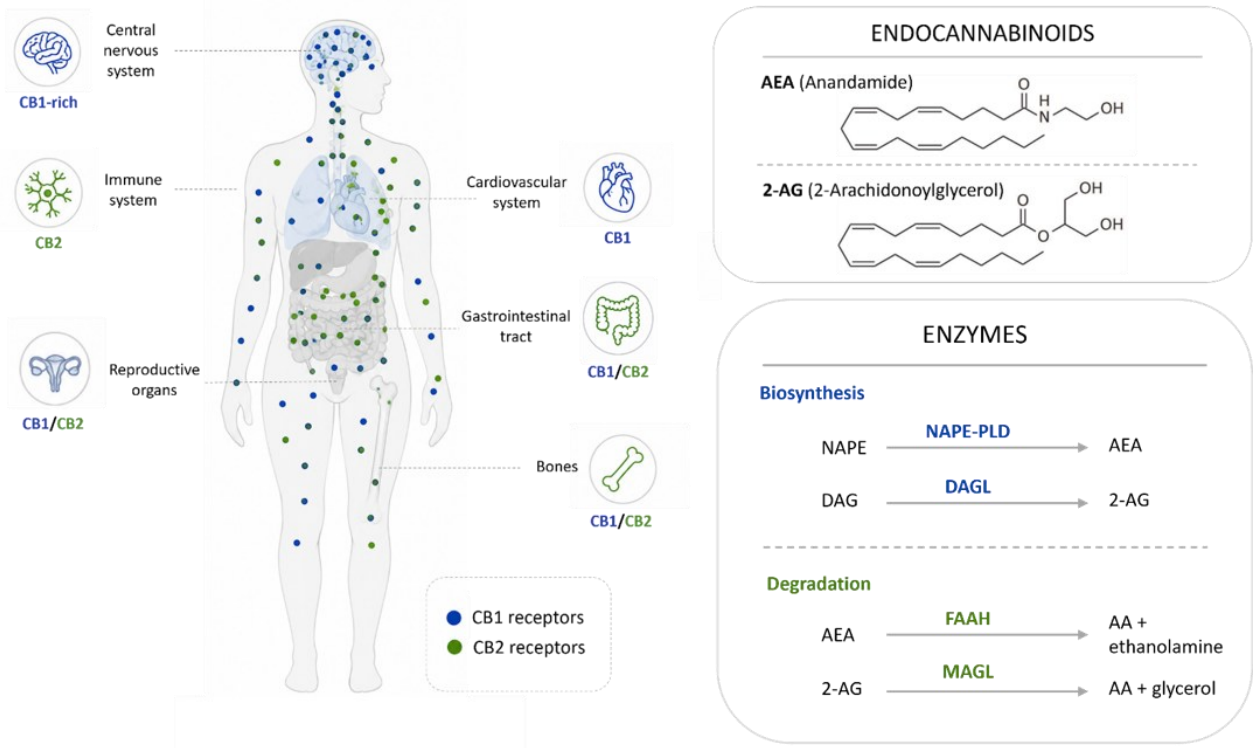

Figure 1 | Tissue distribution and principal mediators and enzymes of the endocannabinoid system. Distribution of cannabinoid receptors CB1 and CB2 across major human organs and tissues, with prominent enrichment of CB1 in the CNS and close association of CB2 with immune and peripheral compartments. Anandamide (AEA) and 2-arachidonoylglycerol (2-AG), the two best-characterized endocannabinoids, are also shown as key endogenous mediators of ECS signaling. The figure also summarizes the main enzymes involved in endocannabinoid biosynthesis and degradation: N-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD) and diacylglycerol lipase (DAGL) contribute to the biosynthesis of AEA and 2-AG, respectively, whereas fatty acid amide hydrolase (*FAAH*) and monoacylglycerol lipase (MAGL) are major enzymes involved in their degradation.

CB2, initially described as an immune-associated receptor, is now also recognized for its involvement in immune cell function, inflammatory responses, and neuroimmune interactions [1,3,4]. The endogenous ligands of these receptors, referred to as endocannabinoids, include anandamide (AEA) and 2-arachidonoylglycerol (2-AG), the two most extensively investigated members of a broader family of bioactive lipid mediators [4]. Unlike classical neurotransmitters, which are synthesized in advance and stored in vesicles, endocannabinoids are mainly produced on demand in response to cellular stimulation (**Figure 2**). This mechanism supports rapid and localized modulation of cellular activity, while also making endocannabinoid activity strongly dependent on tissue context, cellular state and metabolic conditions [4].

Alterations in ECS signaling have been described in a broad range of pathological conditions, including obesity and diabetes, neurodegenerative diseases, such as Alzheimer's disease (AD) and Parkinson's disease (PD), psychiatric conditions, inflammatory, neoplastic and cardiovascular disorders [1,4,5]. However, the functional significance of these alterations remains difficult to define. The ECS does not operate as an isolated signaling pathway but is embedded within broader processes involving lipid metabolism, mitochondrial function, immune signaling, hormonal regulation, and gene-expression networks. Consequently, a variation in a single ECS component may reflect a wider molecular response rather than an isolated defect in endocannabinoid signaling [4].

Changes in endocannabinoid levels may arise from altered biosynthesis or degradation, differences in precursor availability, or remodeling of related lipid pathways [4,6,7]. In cultured bovine adipocytes, for example, modulation of lipolytic pathways was accompanied by altered release of endocannabinoids and related lipid mediators, indicating a close relationship between cellular lipid metabolism and ECS signaling [8]. Receptor-expression data are similarly influenced by the identity and activation state of the cells being studied. In mouse brain, CB2 mRNA has been detected mainly in a small proportion of microglial cells and, to a lesser extent, in neurons, while its expression varied according to the type and timing of the inflammatory stimulus [9]. These findings also illustrate both the value and the limitations of approaches focused on individual molecular targets. Targeted methodologies remain essential for defining receptor function, enzyme activity, and signaling mechanisms, but they may be less suited to evaluating coordinated changes across different molecular levels.

A new conceptual framework is required, capable of capturing the ECS as a dynamic biological network rather than as a collection of independent components. In this context, omics sciences offer a powerful strategy to investigate a biological system across multiple layers of molecular organization. By simultaneously analyzing thousands of molecular features, omics technologies can identify complex molecular patterns associated with physiological regulation and pathological states [10].

In this view, the integration of omics into ECS research does not represent simply a technological improvement, but a paradigm shift. It enables the field to move beyond the isolated study of individual ECS components and toward an understanding of how the broader molecular ecosystem surrounding the ECS contributes to biological outcomes.

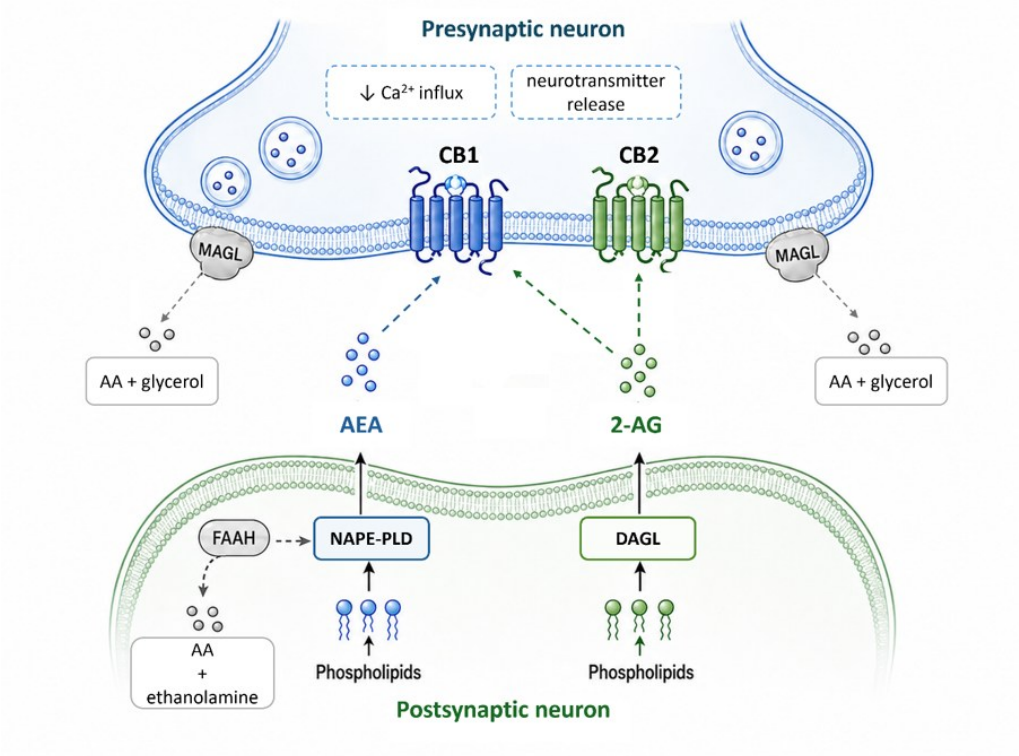

Figure 2 | Retrograde endocannabinoid signaling. Endocannabinoids are produced on demand in the postsynaptic neuron from membrane phospholipid precursors, with AEA mainly generated through NAPE-PLD and 2-AG through DAGL. After their retrograde diffusion across the synaptic cleft, AEA and 2-AG activate presynaptic cannabinoid receptors, primarily CB1, leading to reduced Ca^{2+} influx and neurotransmitter release, particularly at GABAergic and glutamatergic synapses. Signal termination is mediated by enzymatic degradation, mainly through *FAAH* for AEA and MAGL for 2-AG.

2. The Endocannabinoid System and its Multiple Molecular Dimensions

A central feature of the ECS is its context dependence. Rather than operating as a linear signaling cascade with uniform downstream effects, it acts as a modulatory network whose activity is dynamically regulated according to cellular state, tissue microenvironment, physiological and environmental conditions [2,11]. The ECS can be viewed as a regulatory system responsive to both internal physiological states and external influences [2]. Dietary fatty acid intake, stress exposure, gut microbiome composition and aging have been associated with changes in distinct aspects of ECS signaling [12-14]. Although these studies address different dimensions of the ECS regulation, they collectively indicate that endocannabinoid signaling is closely shaped by the biological context in which it operates [12-14]. The close relationship between the ECS and metabolism provides a clear example of this biological context dependence. Endocannabinoids are synthesized from membrane phospholipid-derived precursors, and they are therefore closely connected with cellular lipid metabolism [6]. Changes in nutritional status, fatty acid composition and energy balance can influence endocannabinoid production, while ECS signaling can modulate appetite, adipose-tissue function, insulin sensitivity, and mitochondrial metabolism, creating a bidirectional relationship between metabolic state and ECS signaling [6,11]. This interplay extends beyond systemic metabolic status to the local intestinal environment, where ECS activity is closely interconnected with the gut microbiota. Germ-free mice have displayed marked alterations in intestinal endocannabinoidome signaling across different intestinal regions and developmental stages, supporting a role for microbial composition in the regulation of this lipid network [15]. These findings also suggest that changes in the intestinal ECS should be interpreted in relation to the metabolic and microbial environment in which they occur.

Context-dependent regulation is also evident in immune response. In human LPS-stimulated peripheral blood mononuclear cells, pharmacological activation of CB2 modulated intracellular signaling and cytokine release, providing evidence that ECS signaling can influence inflammatory responses [16]. However, the biological effects of CB2 activation may vary according to cellular state and inflammatory context.

The interaction between the ECS and the nervous system provides a further example of context-specific regulation. Endocannabinoids regulate neurotransmitter release and synaptic plasticity through retrograde signaling mechanisms [17]. Beyond synaptic regulation,

alterations in ECS signaling have also been associated with neuroinflammatory, cognitive and neurodegenerative processes [1,4], as well as with neuropsychiatric disorders [5]. Taken together, these observations highlight the highly interconnected nature of the ECS, whose activity emerges from the interplay between multiple biological processes and is shaped by the molecular and cellular context in which it operates. Capturing this complexity requires approaches capable of examining the ECS beyond individual molecular components and pathways.

3. Omics Technologies as an Integrative Framework for Endocannabinoid Research

The development of high-throughput molecular technologies has changed the way complex biological systems are investigated. Rather than focusing exclusively on predefined molecular targets, omics allow for the simultaneous exploration of multiple molecular layers, including genes, transcripts, proteins, lipids, and metabolites [18]. This multilayered perspective is especially relevant to ECS research, where biological responses cannot be fully interpreted by considering individual components [18,19]. The major omics disciplines provide complementary perspectives:

- **Genomics** may identify either genetic variants affecting ECS components or influencing individual responses to cannabinoid-based interventions.
- **Transcriptomics** can reveal changes in the expression of receptors, enzymes, and signaling-related genes associated with ECS activity.
- **Proteomics** provides information on protein abundance, localization, interactions, and post-translational regulation, thereby linking gene expression to functional molecular mechanisms.
- **Lipidomics** allows for the characterization of endocannabinoids, endocannabinoid-like mediators, lipid precursors, and related bioactive lipid pathways.
- **Metabolomics** can describe the broader small-molecule changes associated with ECS modulation and help connect endocannabinoid signaling with cellular and systemic metabolism.

When integrated, these approaches can reveal coordinated molecular signatures linking ECS-related components to biological pathways and phenotypic outcomes (**Figure 3**). In this sense, multi-omics does not only increase the amount of measurable information but provides a framework for interpreting endocannabinoid signaling within the broader molecular context in which it operates [18,19].

4. Genomics: Understanding the Genetic Basis of Endocannabinoid Regulation

Genome represents a fundamental layer of biological complexity that may influence ECS function and contribute to interindividual variability in the endocannabinoid signaling. Variants in genes encoding cannabinoid receptors, such as *CNR1* and *CNR2*, as well as enzymes involved in endocannabinoid biosynthesis and degradation, including *DAGLA*, *MGLL*, and *FAAH*, have been investigated as potential modifiers of ECS activity and disease-related phenotypes [20,21].

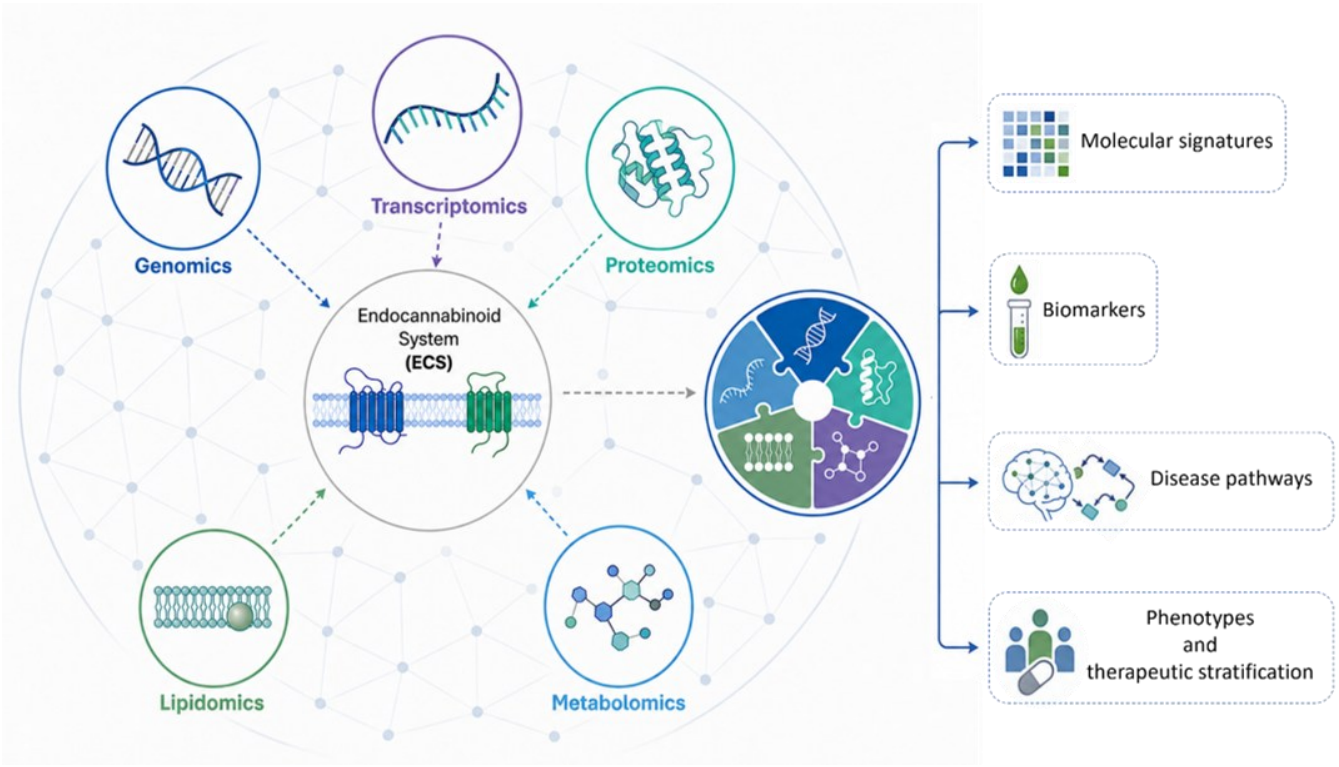

Figure 3 | Multi-omics integration in ECS research.

In a large cohort of individuals undergoing molecular testing for neurological disorders, rare variants in core ECS genes have been analyzed, and significant associations with specific neurological phenotypes were described for *CNR1* and *DAGLA* [20]. Rare *CNR1* variants have been associated with pain sensitivity, particularly migraine/headache, sleep and memory disorders, alone or in combination with anxiety, whereas rare *DAGLA* variants have been associated with seizures and neurodevelopmental disorders, including autism and abnormalities of brain morphology [20]. In parallel, a cross-disorder genome-wide association study (GWAS) meta-analysis has investigated ECS-related genes across psychiatric disorders, including major depressive disorder, bipolar disorder, attention-deficit/hyperactivity disorder, autism spectrum disorder, and schizophrenia, and it has identified *DAGLA* as the strongest gene-level association [21]. These studies suggest that genetic variability within ECS-associated pathways may contribute to neurological and psychiatric disease susceptibility [20,21]. Supporting this concept, expression quantitative trait locus (eQTL) analyses have shown that genetic variation influences the expression of a large proportion of ECS-related genes, with particularly widespread effects on endocannabinoid-catabolizing enzymes and marked tissue-specificity, highlighting a potential genetic contribution to interindividual and tissue-specific variability in ECS regulation [22]. However, these findings should be interpreted with caution, since genetic associations do not necessarily imply causality, and some signals may vary across disorders, cohorts or models applied. Independent replication in well-characterized populations and functional studies is required to clarify whether the identified variants produce measurable effects either on ECS signaling or disease biology [20,21]. Pharmacogenetics represents a promising extension of genomic research in the context of cannabinoid-based therapies. Interindividual differences in therapeutic efficacy, tolerability, and adverse effects may partly reflect genetic variation affecting cannabinoid receptors, endocannabinoid-metabolizing enzymes, and pharmacokinetic pathways involved in cannabinoid absorption, distribution, metabolism, and elimination (ADME) [23]. This is relevant because pharmacogenetic variability may influence both pharmacodynamic targets, such as *CNR1*, *CNR2*, and *FAAH*, and drug-metabolizing systems involved in the metabolism of exogenous cannabinoids [23].

In the future, genomic profiling could contribute to patient stratification and help identify molecular signatures associated with treatment response, tolerability and susceptibility to adverse reactions. However, the current evidence remains preliminary, and genotype-guided approaches for cannabinoid-based therapies have not yet reached a level of validation suitable for routine clinical implementation [23].

Nevertheless, genomics alone cannot capture the full complexity and dynamic nature of ECS regulation. The presence of a genetic variant does not necessarily translate into a measurable functional effect, since its biological consequences need to be interpreted in the broader context of gene expression, protein regulation, and metabolic state [18].

These highlights the need to integrate genomic information with transcriptomic, proteomic, lipidomic and metabolomic data to determine how genetic variation ultimately translates into functional changes in ECS activity.

5. Transcriptomics: Mapping the Cellular Regulation of the Endocannabinoid System

While genomics defines the genetic potential of a biological system, transcriptomics describes how this potential is dynamically expressed under specific physiological, pathological and environmental conditions. The ability to quantify RNA transcripts at a genome-wide scale has greatly expanded the characterization of cellular responses and complex biological networks [24].

In the context of ECS research, transcriptomic approaches enable the investigation of genes encoding cannabinoid receptors, enzymes involved in endocannabinoid biosynthesis and degradation, and molecular components of associated signaling pathways. A relevant contribution of transcriptomic analysis is the identification of cell- and context-specific patterns of ECS regulation [25]. A targeted microfluidic RT-qPCR analysis of astrocytes and microglia, purified from female mice during experimental autoimmune encephalomyelitis (EAE), has revealed both shared and cell-specific alterations in transcripts encoding cannabinoid receptors and enzymes involved in AEA and 2-AG signaling [25]. These changes varied across different stages of EAE, indicating that the transcriptional regulation of ECS components may depend on both cell identity and pathological stage [25]. This example is particularly informative because it shows that glial ECS-related transcriptional changes are not uniform but rather they are shaped by the inflammatory and cellular context in which they occur. Similarly, transcriptomic profiling of human endometrium revealed cycle-dependent changes in multiple genes involved in endocannabinoid synthesis, degradation and transport, further highlighting the dynamic regulation of ECS components according to physiological context [26].

Single-cell RNA sequencing provides an additional level of resolution by examining gene-expression profiles in individual cells rather than averaging signals from heterogeneous cell populations [24]. In the developing mouse paraventricular nucleus of the hypothalamus, the combination of single-cell RNA sequencing together with in situ hybridization and immunohistochemistry has been used to investigate the spatiotemporal distribution of genes and proteins involved in AEA and 2-AG signaling in distinct neuronal subpopulations and astrocytes [27]. This study illustrates the value of transcriptomic approaches for localizing ECS-related components during development, while also supporting the need for complementary molecular analyses to determine whether these components are functionally active [27].

Changes in transcript abundance do not necessarily correspond to changes in protein expression, enzymatic activity and endocannabinoid levels. Transcriptomic findings should be integrated with proteomics, lipidomics, and metabolomics to determine whether transcriptional alterations translate into functional changes in ECS activity [18].

6. Proteomics: Moving from Molecular Expression to Functional Regulation

Although transcriptomics provides information about gene regulation, proteomics offers a complementary and often closer view of the functional state of biological systems. Protein abundance, localization, stability, molecular interactions, and post-translational modifications can influence cellular activity independently of changes in transcript abundance [28].

In ECS research, proteomics can support the investigation of cannabinoid receptors, metabolic enzymes, and associated signaling pathways at the protein level [29]. Specifically, mass spectrometry (MS)-based proteomics enables large-scale identification and quantification of proteins, providing a broad overview of protein-level changes that may accompany ECS regulation or dysregulation [28,29]. Importantly, proteomic approaches can also provide functional information beyond protein abundance. Activity-based protein profiling coupled with chemoproteomics has been used to map the activity of endocannabinoid hydrolases across distinct mouse brain regions, revealing marked region-specific differences in enzymes, such as DAGL-α, FAAH and MAGL [30]. The disagreement observed between enzyme activity and protein abundance further illustrates that transcriptional and proteomic measurements alone may not fully capture ECS function, emphasizing the importance of integrating abundance-based omics with functional activity profiling [30].

Proteomics can also contribute to the characterization of protein–protein interactions [28,31]. This aspect is particularly relevant for cannabinoid receptors, which operate within multiprotein molecular environments containing proteins involved in intracellular signaling, membrane-associated processes, trafficking, and receptor regulation. In this context, affinity purification combined with MS has been used to characterize CB1 receptor-associated proteins in mouse hippocampal glutamatergic neurons and GABAergic interneurons, revealing both shared and cell-type-dependent receptor-associated protein networks [31]. This example illustrates how proteomics can move ECS research beyond the measurement of receptor expression, providing information on the molecular partners that may influence receptor localization, signaling, and functional output.

Specialized proteomics adds a protein-level and functionally informative layer to genomic and transcriptomic data, helping to clarify which ECS-related proteins are present and how they may participate in molecular complexes and cellular networks [28]. In the ECS, this level of information may help clarify how cannabinoid receptors and metabolic enzymes are organized within signaling complexes and how this organization may influence receptor regulation, enzymatic activity, and downstream cellular responses [28,31]. Integration of proteomics with lipidomic and metabolomic data may help determine whether protein-level alterations are accompanied by changes in endocannabinoid availability and downstream metabolic responses, although mechanistic experiments remain necessary to establish causality [18,28].

7. Lipidomics: the Central Role of Lipid Networks in Endocannabinoid Biology

Among the different omics disciplines, lipidomics occupies a particularly central position in ECS research, as endocannabinoids arise from lipid metabolic pathways and function within a broader network of structurally and functionally related mediators [7]. Although AEA and 2-AG remain the most extensively investigated endocannabinoids, their biological significance cannot be fully understood when considered alone. Lipidomics makes it possible to profile these molecules together with their precursors, metabolites, and related bioactive lipids, thereby providing a more integrated view of the lipid networks that shape ECS activity [7,32]. This broader perspective is particularly relevant because endocannabinoid signaling is influenced by substrate and precursor availability, as well as by the activity of interconnected biosynthetic and degradative pathways [7].

Dietary fatty acids can modify the availability of lipid precursors and, consequently, they can influence the production of endocannabinoids and related mediators [6,12]. Omega-3- and omega-6-derived polyunsaturated fatty acids specifically contribute to distinct families of bioactive lipids, including endocannabinoid-related compounds, with potential implications for metabolic and inflammatory regulation [32,33]. In humans, dietary intake of specific fatty acids has been associated with circulating levels of 2-AG and several related N-acylethanolamines and monoacylglycerols, supporting a link between dietary lipid composition and endocannabinoidome profiles [12].

Lipidomics is also particularly informative in inflammatory and inflammation-associated metabolic disorders, where endocannabinoid alterations are not isolated [6,33]. Rather, they may be part of a broader remodeling of lipid mediator pathways, including eicosanoids, specialized pro-resolving mediators, lysoglycerophospholipids, sphingolipids, and other bioactive lipids involved in different phases of inflammatory signaling and resolution [33]. In this context, changes in AEA, 2-AG and related N-acylethanolamines should be interpreted within the surrounding lipid profile, since their biological meaning may depend on the inflammatory and metabolic context in which they occur [33].

MS imaging adds a spatial dimension to lipid analysis by enabling the direct visualization of lipid distributions within tissue sections. This approach has been applied to map the cerebral distribution of 2-AG and to identify region-specific alterations associated with chronic restraint stress [34].

More recently, matrix-assisted laser desorption/ionization–mass spectrometry imaging (MALDI-MSI), particularly MALDI-2, was used to investigate the spatial modulation of major endocannabinoids in a mouse model combining mild traumatic brain injury (mTBI) and AD-related pathology, revealing region- and genotype-dependent changes in AEA, 2-AG and oleoylethanolamide (OEA) levels [35]. Spatially resolved lipid analysis may be particularly valuable in neurological and other complex disorders in which molecular changes are confined to specific anatomical regions or tissue microenvironments.

8. Metabolomics: Connecting ECS Activity with Metabolic Function

While lipidomics focuses on the comprehensive analysis of lipids, metabolomics provides a broader representation of the small-molecule biochemical state of cells, tissues and biological fluids. In the context of the ECS, metabolomics can help characterize the metabolic changes associated with altered endocannabinoid signaling and place variations in their levels within a wider biochemical context [18,36]. This perspective is particularly relevant in metabolic disorders, where ECS dysregulation is unlikely to occur in isolation, but it may be accompanied by coordinated changes across metabolically active tissues and pathways [37,38]. For instance, in a mouse model of diet-induced obesity, high-fat feeding has increased circulating levels of AEA and 2-AG, together with changes in the expression of enzymes involved in endocannabinoid synthesis in white and brown adipose tissue depots [37]. In addition, adipocyte-specific CB1 deletion in mice has shown protection against diet-induced obesity-associated metabolic alterations, reducing adiposity, improving insulin sensitivity, increasing energy expenditure, and promoting adipose-tissue remodeling [38]. These findings indicate that ECS-related metabolic changes should be interpreted considering both circulating lipid mediator profiles and tissue-specific metabolic regulation. In humans, circulating 2-AG levels have been positively associated with triglyceride levels and body fat percentage, supporting a relationship between endogenous cannabinoid signaling and metabolic phenotype [39]. However, these associations do not establish whether altered ECS activity contributes to metabolic dysfunction, or it develops because of it [39]. This distinction is particularly relevant in metabolomic studies, where measured metabolites may represent both drivers and readouts of biological adaptation.

When combined with lipidomic and other omics data, metabolomics may provide a relevant functional correlation between ECS regulation and cellular metabolism [18,36]. A further example is provided by the investigation of serotonergic pathways alongside endocannabinoidome alterations in a mouse model of mTBI and AD-related pathology. MALDI-MSI revealed genotype- and injury-dependent changes in serotonin and related metabolites, highlighting the interplay between neurotransmitter metabolism and ECS-related lipid mediators [40]. This approach illustrates how broader metabolic profiling can expand the interpretation of ECS dysregulation beyond endocannabinoid pathways alone.

Future metabolomic studies should be designed to measure endocannabinoids and related lipid mediators together with the metabolic features that define the phenotype under investigation, rather than treating them as stand-alone circulating markers. Depending on the biological model or clinical cohort, this may include alterations in lipid and glucose metabolism, insulin sensitivity, adipose-tissue activity and inflammatory status. This approach would help clarify whether ECS-related changes reflect a general state of metabolic imbalance or are linked to more specific metabolic alterations and tissue-dependent mechanisms [37-39].

9. Multi-Omics Integration to Interpret ECS Networks

The integration of omics sciences represents one of the most promising directions for the endocannabinoid research, but its value should not be understood simply as the simultaneous collection of larger datasets. In our view, its main contribution is conceptual: by connecting molecular layers that are often investigated separately, multi-omics can help interpret the ECS as a context-dependent regulatory network rather than as a collection of isolated molecular markers. Endocannabinoid signaling emerges from the interaction between genetic background, cell-specific transcriptional programs, protein abundance and activity, lipid mediator availability, metabolic state, cellular identity, and tissue microenvironment. For this reason, the integration of omics approaches may provide a more informative framework than the search for isolated ECS biomarkers alone and may help identify coordinated ECS-related signatures associated with specific physiological states, disease contexts and therapeutic responses [18,41].

This is particularly evident in neurological and neuroinflammatory disorders. In EAE, cell-resolved gene-expression analysis has shown that ECS-related transcriptional changes vary according to glial cell type and disease phase [25]. In human multiple sclerosis lesions, metabolomic profiling, integrated with single-nucleus RNA-sequencing data, linked alterations in lipid metabolism, including endocannabinoid and monoacylglycerol pathways with transcriptomics-based cellular profiles [42]. Other neurological conditions further illustrate the importance of connecting ECS-related alterations with disease heterogeneity and functional consequences. In AD, targeted plasma profiling of oxylipins, endocannabinoids and related lipid mediators in the so-called Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort have revealed changes across disease stages and explored their relationships with sex, apolipoprotein E (APOE) genotype, cognitive performance, and progression from mild cognitive impairment to AD [43]. Although this approach does not represent a complete multi-omics workflow, it shows how ECS-related lipid mediators can be incorporated into stratified molecular analyses of disease heterogeneity [43].

A nice example of an integrated study on the endocannabinoidome has been performed on a mouse model, combining mild traumatic brain injury (mTBI) and AD-related pathology [40]. The integration of targeted lipidomics, gene expression analysis and MALDI-MSI has revealed region- and genotype-dependent alterations, including increased 2-AG levels in the cortex and hippocampus, and linked these changes to alterations in ECS-related enzymes and molecular targets [40]. These findings highlight how multi-omics integration can provide complementary spatial and mechanistic insights into endocannabinoidome dysregulation beyond those achievable with a single omics layer [40]. In rare early-onset PD, biallelic loss-of-function variants in *DAGLB* have been linked to impaired DAGLB-dependent 2-AG synthesis and nigral dopaminergic neuron dysfunction [44]. This genetics-to-function example further illustrates that ECS-related findings become more informative when molecular alterations are

connected to downstream cellular and physiological consequences; however, this evidence should be interpreted within the specific context of DAGLB-associated PD [44].

A similar need for contextual interpretation emerges in metabolic and microbiota-related disorders [6,12,45]. In humans, dietary fatty acid intake and microbiota composition have been associated with circulating profiles of endocannabinoids and related lipid mediators [12]. In genetically obese and diabetic mice, targeted lipidomics and qPCR-based transcriptomics have revealed intestinal segment-specific alterations in endocannabinoidome and oxylipin signaling, together with changes in inflammatory markers and gut microbiota composition [45]. These observations support the idea that ECS-related lipid networks should be interpreted within a broader host–microbiota-metabolism axis, rather than as isolated metabolic readouts [45].

Cancer represents another context in which the biological meaning of ECS signaling strongly depends on cellular composition and microenvironmental conditions. ECS components may be expressed by both tumor and immune cells, and their association with cancer progression can vary according to tumor type and immune context [46]; therefore, a universal pro- or antitumor role of ECS activation cannot be assumed [46]. In melanoma, the combined analysis of The Cancer Genome Atlas (TCGA) bulk tumor gene-expression data, a previously published single-cell RNA-sequencing dataset, and RNAscope on human tissue sections has shown that *CNR2* expression is predominantly associated with tumor-infiltrating B cells [47]. In a murine melanoma model, Cnr2 deficiency has been associated with enhanced tumor growth, impaired differentiation and activation of tumor-infiltrating B cells, increased regulatory T-cell frequencies, and reduced CD8+ T-cell infiltration [47].

Taken together, these examples indicate that multi-omics integration can help move ECS research from the description of individual molecular alterations toward a more context-aware interpretation of disease mechanisms and therapeutic variability [18,41].

10. Challenges and Future Perspectives: Toward a System-Level Understanding of the ECS

Despite the considerable potential of omics-based research, several challenges must be addressed before these approaches can provide a comprehensive and clinically useful understanding of the ECS. Multi-omics studies generate high-dimensional and heterogeneous datasets that differ in molecular coverage, analytical sensitivity, scale, and preprocessing requirements, while missing values, batch effects, and platform-specific biases further complicate their integration and interpretation [18,41,48]. Robust experimental design, standardized procedures for sample collection and analysis, and adequate statistical analysis are essential to improve reproducibility across studies [18,41]. Computationally identified associations should also be independently replicated and validated through targeted biochemical analyses, cellular models and functional perturbation experiments before they can be interpreted as causal mechanisms [18,41,48]. These requirements are especially relevant to the ECS, whose molecular organization is highly dynamic and varies across tissues, cell types, physiological states, metabolic and environmental contexts [1,2,11].

Artificial intelligence, statistical learning and network-based modelling may facilitate multi-omics integration, missing-data handling, and the identification of complex relationships across molecular layers. Their reliability will nevertheless depend on data quality, adequate sample size, appropriate model selection, interpretability, and independent validation [48]. Omics technologies should therefore complement, rather than replace, classical molecular and functional approaches.

The future of endocannabinoid research is unlikely to depend solely on the discovery of additional molecular components, but increasingly on the ability to understand how established and emerging elements of the ECS interact across biological scales. This transition may improve the identification of disease mechanisms, molecular signatures and therapeutic opportunities and support more precise patient stratification and ECS-targeting strategies.

References

[1] M. Şerban, C. Toader, R.A. Covache-Busuioc, Int. J. Mol. Sci. 26 (2025) 11132. DOI:10.3390/ ijms262211132

[2] H.C. Lu, K. Mackie, Biol. Psychiatry Cogn. Neurosci. Neuroimaging 6 (2021) 607–615. DOI: 10.1016/j.bpsc.2020.07.016

[3] A. Varghese, J. Liu, W. Guo, T.A.A. Patterson, H. Hong, Front. Chem. Biol. 5 (2026) 1830007. DOI: 10.3389/fchbi.2026.1830007

[4] P. Simankowicz, J. Stępniewska, J. Clin. Med. 14 (2025) 2851. DOI: 10.3390/jcm14082851

[5] T. Mušić, T.L. Turnšek, Biomedicines 14 (2026) 968. DOI: 10.3390/biomedicines14050968

[6] M.M. Almeida, C.P. Dias-Rocha, C. Calviño, I.H. Trevenzoli, Mol. Cell. Endocrinol. 542 (2022) 111522. DOI: 10.1016/ j.mce.2021.111522

[7] J. Keereetaweep, K.D. Chapman, Neural Plast. 2016 (2016) 2426398. DOI: 10.1155/2016/2426398

[8] M.N. Myers, M. Chirivi, J.C. Gandy, J. Tam, M. Zachut, G.A. Contreras, J. Anim. Sci. Biotechnol. 15 (2024) 103. DOI: 10.1186/ s40104-024-01062-z

[9] W. Grabon, A. Ruiz, N. Gasmi, C. Degletagne, B. Georges, A. Belmeguenai, J. Bodennec, S. Rheims, G. Marcy, L. Bezin, J. Neuroinflammation 21 (2024) 206. DOI: 10.1186/s12974-024-03202-8

[10] M. Zarid, J. Genome Biotechnol. Genet. 1 (2026) 8. DOI: 10.3390/jgbg1020008

[11] A. Busquets-García, J.P. Bolaños, G. Marsicano, Nat. Metab. 4 (2022) 848–855. DOI: 10.1038/s42255-022-00600-1

[12] S. Castonguay-Paradis, S. Lacroix, G. Rochefort, L. Parent, J. Perron, C. Martin, B. Lamarche, F. Raymond, N. Flamand, V. Di Marzo, A. Veilleux, Sci. Rep. 10 (2020) 15975. DOI: 10.1038/s41598-020-72861-3

[13] A. Demaili, A. Portugalov, M. Maroun, I. Akirav, K. Braun, J. Bock, Front. Cell. Neurosci. 18 (2024) 1474992. DOI: 10.3389/fncel.2024.1474992

[14] Y. Park, B.A. Watkins, Vitam. Horm. 115 (2021) 129–172. DOI: 10.1016/bs.vh.2020.12.007

[15] C. Manca, B. Boubertakh, N. Leblanc, T. Deschênes, S. Lacroix, C. Martin, A. Houde, A. Veilleux, N. Flamand, G.G. Muccioli, F. Raymond, P.D. Cani, V. Di Marzo, C. Silvestri, J. Lipid Res. 61 (2020) 70–85. DOI: 10.1194/jlr.ra119000424

[16] A. Capozzi, D. Caissutti, V. Mattei, F. Gado, S. Martellucci, A. Longo, S. Recalchi, V. Manganelli, G. Riitano, T. Garofalo, M. Sorice, C. Manera, R. Misasi, Molecules 27 (2022) 64. DOI: 10.3390/molecules27010064

[17] B.L. Winters, C.W. Vaughan, Neuropharmacology 197 (2021) 108736. DOI: 10.1016/j.neuropharm.2021.108736

[18] M.A. Wörheide, J. Krumsiek, G. Kastenmüller, M. Arnold, Anal. Chim. Acta 1141 (2021) 144–162. DOI: 10.1016/j.aca.2020.10.038

[19] P. Berman, L. Sulimani, A. Gelfand, K. Amsalem, G.M. Lewitus, D. Meiri, Talanta 219 (2020) 121336. DOI: 10.1016/j.talanta.2020.121336

[20] D.R. Smith, C.M. Stanley, T. Foss, R.G. Boles, K. McKernan, PLoS One 12 (2017) e0187926. DOI: 10.1371/journal.pone.0187926

[21] H.K. Kim, V.F. Gonçalves, M.I. Husain, D.J. Müller, B.H. Mulsant, G. Zai, S. Kloiber, Psychiatry Res. 330 (2023) 115563. DOI: 10.1016/j.psychres.2023.115563

[22] K. Tanaka, A.A. Amoako, S. Mortlock, P.A.W. Rogers, S.J. Holdsworth-Carson, J.F. Donoghue, W.T. Teh, G.W. Montgomery, B. McKinnon, J. Cannabis Res. 7 (2025) 29. DOI: 10.1186/s42238-025-00275-x

[23] S. Hryhorowicz, M. Walczak, O. Zakerska-Banaszak, R. Słomski, M. Skrzypczak-Zielińska, Eur. J. Drug Metab. Pharmacokinet. 43 (2018) 1–12. DOI: 10.1007/s13318-017-0416-z

[24] R. Stark, M. Grzelak, J. Hadfield, Nat. Rev. Genet. 20 (2019) 631–656. DOI: 10.1038/s41576-019-0150-2

[25] Á. Moreno-García, A. Bernal-Chico, T. Colomer, A. Rodríguez-Antigüedad, C. Matute, S. Mato, Biomolecules 10 (2020) 1228. DOI: 10.3390/biom10091228

[26] K. Tanaka, A.A. Amoako, S. Mortlock, P.A.W. Rogers, S.J. Holdsworth-Carson, J.F. Donoghue, W.T. Teh, G.W. Montgomery, B. McKinnon, Sci. Rep. 12 (2022) 9400. DOI: 10.1038/s41598-022-13488-4

[27] E.O. Tretiakov, Z. Hevesi, C. Böröczky, A. Alpár, T. Harkany, E. Keimpema, Cells 14 (2025) 788. DOI: 10.3390/cells14110788

[28] R. Aebersold, M. Mann, Nature 537 (2016) 347–355. DOI: 10.1038/nature19949

[29] G. Seabra, A.C.B. Falvella, P.C. Guest, D. Martins-de-Souza, V. de Almeida, Proteomics 18 (2018) e1700270. DOI: 10.1002/pmic.201700270

[30] M.P. Baggelaar, A.C.M. van Esbroeck, E.J. van Rooden, B.I. Florea, H.S. Overkleeft, G. Marsicano, F. Chaouloff, M. van der Stelt, ACS Chem. Biol. 12 (2017) 852–861. DOI: 10.1021/acschembio.6b01052

[31] T. Mattheus, K. Kukla, T. Zimmermann, S. Tenzer, B. Lutz, J. Proteome Res. 15 (2016) 3585–3601. DOI: 10.1021/acs.jproteome.6b00339

[32] W. Röhrig, S. Achenbach, B. Deutsch, M. Pischetsrieder, J. Lipid Res. 60 (2019) 1475–1488. DOI: 10.1194/jlr.d094680

[33] V. Chiurchiù, A. Leuti, M. Maccarrone, Front. Immunol. 9 (2018) 38. DOI: 10.3389/fimmu.2018.00038

[34] Q. Zhai, A. Islam, B. Chen, H. Zhang, D.H. Chi, M. Al Mamun, Y. Takahashi, N. Sato, H. Yamasue, Y. Nakajima, Y. Nagashima, F. Sano, T. Sato, T. Kahyo, M. Setou, Cells 12 (2023) 393. DOI: 10.3390/cells12030393

[35] E. Salviati, F. Guida, D. La Gioia, F. Merciai, S. Maione, V. Di Marzo, P. Campiglia, F. Piscitelli, E. Sommella, Talanta 290 (2025) 127811. DOI: 10.1016/j.talanta.2025.127811

[36] T. Jendoubi, J. Engel, Metabolites 11 (2021) 184. DOI: 10.3390/metabo11030184

[37] E.N. Kuipers, V. Kantae, B.C.E. Maarse, S.M. van den Berg, R. van Eenige, K.J. Nahon, A. Reifel-Miller, T. Coskun, M.P.J. de Winther, E. Lutgens, S. Kooijman, A.C. Harms, T. Hankemeier, M. van der Stelt, P.C.N. Rensen, M.R. Boon, Front. Physiol. 9 (2019) 1913. DOI: 10.3389/fphys.2018.01913

[38] I.R. De Azua, G. Mancini, R.K. Srivastava, A.A. Rey, P. Cardinal, L. Tedesco, C.M. Zingaretti, A. Sassmann, C. Quarta, C. Schwitter, A. Conrad, N. Wettschureck, V.K. Vemuri, A. Makriyannis, J. Hartwig, M. Mendez-Lago, L. Bindila, K. Monory, A. Giordano, S. Cinti, G. Marsicano, S. Offermanns, E. Nisoli, U. Pagotto, D. Cota, B. Lutz, J. Clin. Invest. 127 (2017) 4148–4162. DOI: 10.1172/jci83626

[39] V. Kantae, K.J. Nahon, M.E. Straat, L.E.H. Bakker, A.C. Harms, M. Van Der Stelt, T. Hankemeier, I.M. Jazet, M.R. Boon, P.C.N. Rensen, Sci. Rep. 7 (2017) 7558. DOI: 10.1038/s41598-017-07980-5

[40] F. Guida, M. Iannotta, A. Lauritano, R. Infantino, E. Salviati, R. Verde, L. Luongo, E.M. Sommella, F.A. Iannotti, P. Campiglia, S. Maione, V. Di Marzo, F. Piscitelli, Acta Neuropathol. Commun. 12 (2024) 113. DOI: 10.1186/s40478-024-01820-0

[41] S. Graw, K. Chappell, C.L. Washam, A. Gies, J. Bird, M.S. Robeson, S.D. Byrum, Mol. Omics 17 (2021) 170–185. DOI: 10.1039/d0mo00041h

[42] D.C. Ladakis, E. Pedrini, M.I. Reyes-Mantilla, M. Sanjayan, M.D. Smith, K.C. Fitzgerald, C.A. Pardo, D.S. Reich, M. Absinta, P. Bhargava, Neurol. Neuroimmunol. Neuroinflamm. 11 (2024) e200219. DOI: 10.1212/nxi.0000000000200219

[43] K. Borkowski, C. Yin, A. Kindt, N. Liang, E. de Lange, C. Blach, J.W. Newman, R. Kaddurah-Daouk, T. Hankemeier, Alzheimer's Res. Ther. 18 (2026) 21. DOI: 10.1186/s13195-025-01939-9

[44] Z. Liu, N. Yang, J. Dong, W. Tian, L. Chang, J. Ma, J. Guo, J. Tan, A. Dong, K. He, J. Zhou, R. Cinar, J. Wu, A.G. Salinas, L. Sun, M. Kumar, B.T. Sullivan, B.B. Oldham, V. Pitz, M.B. Makarious, J. Ding, J. Kung, C. Xie, S.L. Hawes, L. Wang, T. Wang, P. Chan, Z. Zhang, W. Le, S. Chen, D.M. Lovinger, C. Blauwendraat, A.B. Singleton, G. Cui, Y. Li, H. Cai, B. Tang, Nat. Commun. 13 (2022) 3495. DOI: 10.1038/s41467-022-31168-9

[45] F. Suriano, C. Manca, N. Flamand, M. Van Hul, N.M. Delzenne, C. Silvestri, P.D. Cani, V. Di Marzo, Cells 12 (2023) 411. DOI: 10.3390/cells12030411

[46] M. Kienzl, J. Kargl, R. Schicho, Int. J. Mol. Sci. 21 (2020) 8929. DOI: 10.3390/ijms21238929

[47] T. Gruber, S. Robatel, M. Kremenovic, L. Bäriswyl, J. Gertsch, M. Schenk, Cancers 13 (2021) 1934. DOI: 10.3390/cancers13081934

[48] J.E. Flores, D.M. Claborne, Z.D. Weller, B.J.M. Webb-Robertson, K.M. Waters, L.M. Bramer, Front. Artif. Intell. 6 (2023) 1098308. DOI: 10.3389/frai.2023.1098308