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REVIEW

Developing Peripheral Biochemical Biomarkers of Brain Disorders: Insights from Zebrafish Models

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Abstract—High prevalence of human brain disorders necessitates development of the reliable peripheral biomarkers as diagnostic and disease-monitoring tools. In addition to clinical studies, animal models markedly advance studying of non-brain abnormalities associated with brain pathogenesis. The zebrafish (*Danio rerio*) is becoming increasingly popular as an animal model organism in translational neuroscience. These fish share some practical advantages over mammalian models together with high genetic homology and evolutionarily conserved biochemical and neurobehavioral phenotypes, thus enabling large-scale modeling of human brain diseases. Here, we review mounting evidence on peripheral biomarkers of brain disorders in zebrafish models, focusing on altered biochemistry (lipids, carbohydrates, proteins, and other non-signal molecules, as well as metabolic reactions and activity of enzymes). Collectively, these data strongly support the utility of zebrafish (from a systems biology standpoint) to study peripheral manifestations of brain disorders, as well as highlight potential applications of biochemical biomarkers in zebrafish models to biomarker-based drug discovery and development.

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INTRODUCTION

Central nervous system (CNS) disorders, such as neurodegenerative (Alzheimer's and Parkinson's) and affective (depression and anxiety) diseases, are complex, wide-spread, and treatment-resistant, and they contribute significantly to the global public health costs [1, 2]. The lack of objective evidence-based approaches, especially for early diagnostics of these disorders, underlies the growing interest to studying biomarkers of the CNS pathogenesis, including behavioral, morphological, and molecular responses.

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In addition to molecular biomarkers in the brain, pathological biochemical changes also occur peripherally, necessitating identification of novel biochemical biomarkers of CNS disorders [3, 4].

Studying clinical biomarkers *in vivo* is often hindered by impossibility of measuring pathological markers directly in the brain tissue using invasive methods. Thus, using pathological biomarkers from peripheral samples, such as blood or skin, becomes more relevant [3, 5]. Validity of this approach is based on the fact that various signs of central pathology can be successfully measured in peripheral tissues. For example, while this is particularly common for the diseases associated with neuroendocrine deficits or with damaged blood-brain barrier (BBB) [6], peripheral biomarkers can originate in the periphery, reflecting systemic nature of CNS pathogenesis [7, 8].

In addition to the established clinical biomarkers, there are also animal models, especially rodents, widely used to study CNS pathogenesis and biomarker validation [9-11]. Because collecting brain tissue is not a problem in animals, their peripheral biomarkers associated with central neuropathology are relatively understudied. However, validity of the peripheral biomarkers in animals would be markedly reinforced by showing that they reproduce the signs of peripheral dysregulations observed in clinical studies, e.g., endocrine hyperactivation in animal models of depression [11, 12]. Moreover, animal models of CNS diseases can help not only to identify peripheral biomarkers among those already discovered in patients, but also to search for novel biomarkers not yet identified previously [13]. Thus, studying non-brain abnormalities in animal models of CNS pathologies helps elucidate their pathophysiological mechanisms, providing novel insights into a complex 'systems biology' nature of these disorders, eventually facilitating translational research and development of novel therapies [14, 15].

Addressing this problem further, here we review mounting evidence on peripheral biomarkers of brain disorders, focusing on altered biochemistry (lipids, carbohydrates, proteins, and other non-signal molecules, as well as metabolic reactions and activity of enzymes), in a novel model organism, the zebrafish (*Danio rerio*). Zebrafish is becoming one of the most important model species in biomedicine, including neuroscience [16-20]. The idea for the present paper originated from the invited talk on this topic, given by the senior co-author in May 2022, and subsequent discussion, at Professor Vladimir P. Skulachev's seminar at Belozersky Institute of Physico-Chemical Biology in Lomonosov Moscow State University. This paper is dedicated to the memory of academician V. P. Skulachev (1935-2023), a brilliant scientist, a respected scholar, and a great colleague.

ZEBRAFISH MODELS AND BIOMARKERS OF CNS DISORDERS

A common problem of using animal biomarkers in biomedical research is their ambiguous interpretation and often somewhat unclear validity [21]. For example, this problem is particularly inherent in translational neuroscience since various animal models may have their own, acquired through natural evolution, unique characteristics [22]. Thus, one of the strategies for a more accurate and comprehensive modeling of CNS pathobiology is to expand the number of model objects (beyond the traditionally used rodents), thereby utilizing a wider spectrum of animals from various taxa [22-24]. A small teleost fish, the zebrafish, has recently emerged as a novel powerful model organism in translational neuroscience [25, 26]. This fish possesses several key advantages over other model organisms (e.g., rodents), providing a reasonable compromise between the system complexity and practical simplicity [27], and also enabling a large-scale modeling of "core", evolutionary conserved aspects of complex brain disorders [28, 29]. Table 1 summarizes both advantages and limitations of zebrafish models in biomedical research (also see further).

Multiple zebrafish models of brain disorders have also been developed (Tables 2 and 3), based on targeting a wide range of their CNS biomarkers. For example, similarly to rodent studies, zebrafish models of Parkinson's disease utilize reduced brain levels of dopamine and tyrosine hydroxylase (the main enzyme of dopamine biosynthesis) as markers of dopaminergic neuronal loss [43, 44]. Likewise, in the zebrafish models of pentylenetetrazol (PTZ)-induced epilepsy, the brain gene expression level of *c-fos* is often used as a biomarker of overall neuronal activity [45, 46]. Another example involves biomarkers of neuroinflammation observed in various zebrafish models of traumatic brain injury [47, 48]. While multiple other (e.g., endocrine, genomic, and proteomic) peripheral biomarkers of CNS disorders have already been developed as well, here we focus on purely biochemical biomarkers, including lipids, non-signaling proteins, carbohydrates, amino acids and their derivatives that can be easily analyzed in peripheral samples. Accordingly, description of the peripheral genomic (mutations and genetic polymorphism), transcriptomic (genes expression

Abbreviations: ATP, adenosine triphosphate; CNS, central nervous system; EEG, electroencephalography; GABA, γ-aminobutyric acid; NPC, Niemann–Pick type C; PLP, pyridoxal 5′-phosphate; PLPBP, PLP-binding protein; PNPO, pyridoxamine 5′-phosphate oxidase.

Table 1. Brief summary of selected major advantages and limitations of utilizing zebrafish models in translational biomedicine and neuroscience

Advantages	References
Low cost of maintenance	[30]
Rapid development	[25]
Highly tractable genome	[31]
High percent of genetic homology to humans	[32]
Ease of pharmacological manipulations	[30]
Ease of genetic manipulations	[33]
Potential for high-throughput drug screening	[34]
Limitations	
Evolutionary remoteness from humans, contributing to some existing genetic, biochemical, and physiological differences	[35, 36]
Certain neuroanatomical differences from mammals (e.g., lack of neocortex and midbrain dopaminergic neurons)	[16, 37, 38]
Some of the genes exist in two copies compared to one copy in mammals (due to the teleost-specific whole-genome duplication event)	[39]
Limited availability of the genetically characterized inbred and outbred strains compared to rodent models	[26, 40]
Superior neuroregenertative potential (vs. mammals) that complicates modeling neurodegenerative disorders	[41]
Lack of certain complex behaviors characteristic of mammals (e.g., parental care)	[42]

Table 2. Selected examples of zebrafish models of human brain disorders and their CNS biomarkers

Models	References
Parkinson's disease	
↓ Tyrosine hydroxylase	[43]
↓ Dopamine	[44]
Epilepsy	
↓ *c-fos* expression	[45, 46]
↓ γ-Aminobutyric acid (GABA)	[49]
Stress-related (affective) disorders	
↓ Glucocorticoid receptor expression	[50]
↓ Serotonin	[51]

levels), and endocrine (hormones, cytokines) biomarkers was beyond the scope of the present study.

PERIPHERAL BIOCHEMICAL BIOMARKERS OF ZEBRAFISH CNS PATHOGENESIS

Stress-related disorders. Stress is a key risk factor in pathogenesis of multiple CNS diseases, including anxiety and depression – the two most common and widespread brain maladies [76-78]. There are many zebrafish models based both on acute [79-82] and chronic stress exposure [50, 83-89] that recapitulate the signs of central and peripheral dysregulation characteristic of stress-related pathologies in humans. While cortisol is widely accepted as a peripheral biomarker of stress level in zebrafish [90-92], metabolomic analyses may also accurately assess the overall physiological stress response of an organism [52].

Indeed, fish subjected to acute netting stress and two different behavioral tests demonstrated changes

Table 3. Zebrafish models of human brain disorders and their peripheral biochemical biomarkers

Models, biomarkers	Responses	References
Stress-related disorders		
Alanine, taurine, adenosine, creatine, lactate, acetate, leucine/isoleucine, and histidine		[52]
HADHB, hspa8, hspa5, actb1, mych4, atp2a1, zgc:86709, and zgc:86725 proteins	↑	[53]
Heart proteins of glucose metabolism, gluconeogenesis, the ubiquitin–proteasome system and peroxisomal proliferator-activated receptor signaling	↓ rate of synthesis	[54]
Niemann–Pick type C disease (NPC)		
Unesterified cholesterol	↑	[55-59]
Phospholipids and sphingolipids	altered whole-body levels	[60]
Epilepsy (metabolic biomarkers)		
Glycolysis rate and mitochondrial respiration rate	↓	[61-63]
Basal respiration, maximal respiration, mitochondrial respiration, proton leaks, and ATP-linked respiration rates	↑	[64]
Glucose level	↓	[61]
B6-responsive epilepsies		
Pyridoxal and pyridoxal 5′-phosphate	↓	[65-68]
Pyridoxine-dependent epilepsy		
Piperideine-6-carboxylate, aminoadipate semialdehyde, pipecolic acid	↑	[67, 68]
The Krebs cycle metabolites (citrate, malate, fumarate, isocitrate, lactate)	↓	[69]
γ-Aminobutyric acid (GABA) pathway metabolites (γ-hydroxybutyrate, glutamine, glutamate, total GABA, succinic semialdehyde)	↓	[69]
Activity of electron transport chain enzymes	↓	[69]
Pyridoxamine 5′-phosphate oxidase (PNPO) deficiency		
Pyridoxamine 5′-phosphate, pyridoxine 5′-phosphate	↑	[65]
Glycine, glutamate and GABA	↓	[65]
Lysine, arginine, tryptophan, methionine, phenylalanine, and threonine	↑	[65]
Pyridoxal 5′-Phosphate (PLP)-Binding Protein (PLPBP) deficiency		
Lysine, threonine, asparagine, glutamate, glutamine, proline, alanine, α-aminobutyric acid, GABA	↓	[66]
Methionine, cystathionine, isoleucine, tyrosine, β-alanine, phenylalanine, aminoisobutyric acid, tryptophan	↑	[66]

Table 3 (*cont.*)

Models, biomarkers	Responses	References
Parkinson's disease		
Dehydrogenase-dependent resazurin metabolism	↓	[70]
Maximal mitochondrial respiration	↓	[71]
Parkin, pink1, and dj-1 protein content	↓	[72]
Lipid peroxidation	↑	[73, 74]
Glutathione	↓	[73, 75]
Superoxide dismutase activity	↓	[73, 74]
Glutathione-S-transferase and catalase activity	↓	[73, 74]

in the whole-body quantitative content of a number of metabolites (e.g., alanine, taurine, adenosine, creatine, lactate, acetate, leucine/isoleucine, and histidine). Importantly, content of some metabolites seemed to be influenced not only by the netting stress exposure *per se*, but also by behavioral testing procedures – which themselves are well-established stressors – indicating the sensitivity of metabolomic characterization to biochemical alterations induced by altered environment [52]. Another proteomic study has demonstrated that chronic stress (electric shock) upregulates the whole-body content of several zebrafish proteins (e.g., HADHB, hspa8, hspa5, actb1, mych4, atp2a1, zgc:86709, and zgc:86725) regardless of stress predictability [53]. Besides the heat shock proteins, these protein biomarkers include enzymes, cation transporters, cytoskeleton proteins, increased level of which may be considered to play a role in counteracting conditions associated with elevated fear/anxiety levels.

Moreover, in addition to CNS, cardiovascular system is also highly susceptible to the destructive effects of stress [93, 94]. Proteomic analyses in the chronic unpredictable stress (CUS) zebrafish model revealed changes caused by stress in the zebrafish hearts [95]. For example, the observed anxiety-like behavioral phenotype was accompanied by significantly altered synthesis rates of several heart proteins involved in such processes as glycolysis, gluconeogenesis, and hypoxia response. Notably, even acute low-level stress affects zebrafish heart proteome, causing decrease in the rates of degradation of cardiac muscle proteins [54].

Niemann–Pick type C disease. The Niemann–Pick type C disease (NPC) is a severe autosomal recessive disease caused by mutations in the *NPC1* and *NPC2* genes that result in abnormal lysosomal trafficking of cholesterol and some other lipids [96-98]. This disease is characterized by a wide range of progressive neurological symptoms including neurodegeneration [99, 100]. Recently, several genetic zebrafish models of NPC have been successfully developed targeting both *NPC1* [55, 56, 59] and *NPC2* [57, 58] orthologs of the respective human genes. Besides the characteristic NPC-like neurological symptoms and visceral organs damage, these fish models recapitulate the key biochemical hallmark of the disease – intracellular accumulation of unesterified cholesterol. Importantly, free cholesterol accumulates in the cells of almost all body tissues of zebrafish and its presence can be determined by simple staining techniques [55, 56, 59]. Treatment of larval zebrafish with 2HPβCD (a drug effective in clinical trials) significantly reduces the cholesterol-positive staining in neuromast cells [59]. Collectively, this supports utility of unesterified cholesterol as a suitable biomarker of NPC in zebrafish, potentially applicable for high-throughput drug screening. However, cholesterol is not the only lipid dysregulated in NPC; altered whole-body distribution of several sphingolipids and phospholipids in the *npc1*$^{-/-}$ zebrafish has been revealed in a recent study using elaborated 3D MALDI mass spectrometry imaging (MALDI-MSI) spectrometry imaging [60]. Thus, unique lipid biosignatures for different organs could shed light on the nature of multifaceted pathological manifestations of NPC.

Epilepsy. Epilepsy is a group of severely debilitating neurological disorders characterized by spontaneous and recurrent seizures. Zebrafish is becoming increasingly popular as an alternative model organism in epilepsy research, with multiple pharmacological (using proconvulsant drugs) and genetic (based on epilepsy-causing mutations) models of epilepsy in zebrafish being developed (for comprehensive review see [101]). While most of these models employ behavioral (seizure-like behavior) and electroencephalographic (EEG) biomarkers, some studies indicate systemic bio-

chemical/bioenergetic dysregulation that accompanies the observed epilepsy phenotype.

Indeed, the use of biochemical biomarkers is common in zebrafish models of B6-responsive epilepsies – a group of rare genetic disorders, characterized by severe neonatal seizures that can be alleviated by vitamin B6 [66, 102, 103]. For example, *aldh7a1*$^{-/-}$ zebrafish larvae represent the first animal model of pyridoxine-dependent epilepsy, as they readily recapitulate the main biochemical markers of this disorder: accumulation of piperideine-6-carboxylate, aminoadipate semialdehyde (lysine catabolism metabolites, the substrates of ALDH7a1 enzyme) and reduced levels of B6 vitamers – pyridoxal (PL), pyridoxal 5′-phosphate (PLP), and pyridoxamine 5′-phosphate (PMP) [67, 68]. Additionally, these fish exhibit an approximately twice lower systemic levels of γ-aminobutyric acid (GABA) [67]. While B6 deficiency in the pyridoxine-dependent epilepsy is known to be caused by chemical complexation of PLP with piperideine-6-carboxylate [104], the proposed explanation of reduced GABA levels suggests impaired activity of PLP-dependent glutamate decarboxylase – the key enzyme of GABA biosynthesis [67]. A broad spectrum of biochemical dysregulations in *aldh7a1*$^{-/-}$ zebrafish likely involves reduced levels of tricarboxylic acid (Krebs) cycle metabolites, GABA pathway metabolites, and activity of the electron transport chain enzymes [69].

Biochemical biomarkers have also been extensively characterized in the zebrafish model of pyridoxamine 5′-phosphate oxidase (PNPO) deficiency – a B6-responsive epilepsy caused by mutation in the gene encoding the PLP biosynthesis enzyme [105]. The UPLC-MS/MS analyses of extracts of homogenized bodies of *pnpo*$^{-/-}$ zebrafish reveal the accumulation of pyridoxamine 5′-phosphate, pyridoxine 5′-phosphate (substrates of PNPO), and reduced levels of pyridoxal 5′-phosphate and pyridoxal (PNPO downstream products) [65]. Moreover, the *pnpo*$^{-/-}$ zebrafish display reduced levels of glycine, glutamate, and GABA, and elevated levels of several essential amino acids (lysine, arginine, tryptophan, methionine, phenylalanine, and threonine), suggesting impaired activity of the PLP-dependent enzymes involved in their metabolism [65]. In contrast, the PLP treatment normalizes aberrant behavior of *pnpo*$^{-/-}$ zebrafish, increases fish survival, and restores the levels of glycine, glutamate, and GABA, although accumulation of pyridoxamine (PM), PMP and essential amino acids persisted [65, 106].

The PLP-binding protein (PLPBP) deficiency is another B6-responsive epileptic disorder that has been successfully modeled in zebrafish. The exact function of PLPBP and etiology of this recently described disorder remain unclear [107]. Similarly to human patients, *plpbp*$^{-/-}$ zebrafish mutants display reduced systemic concentrations of PLP, pyridoxal, lysine, threonine, asparagine, glutamate, glutamine, proline, alanine, α-aminobutyric acid, GABA, and increased concentrations of methionine, cystathionine, isoleucine, tyrosine, β-alanine, phenylalanine, aminoisobutyric acid, and tryptophan [66]. Overall, zebrafish genetic models of B6-responsive epilepsies display a wide range of biochemical alterations, most of which can be explained by impaired activity of the B6-dependent enzymes. These biochemical markers support the ability of these models to accurately recapitulate human diseases and can serve as easily accessible tools for the model validation. Moreover, these findings provide valuable insights into pathophysiology of the B6-responsive epilepsies, corroborating, for example, low GABA levels as a possible mechanism of epileptogenesis.

Mounting evidence supports the link between human epilepsy and multiple metabolic defects, including changes in glucose metabolism, mitochondrial respiration, and glycolysis [108, 109]. Metabolism is also dysregulated in the zebrafish models of epilepsy [61-64]. For example, in a zebrafish model of Dravet syndrome (epilepsy caused by mutations in the sodium NaV channel), typical behavioral and EEG changes are accompanied by the decreased rates of mitochondrial respiration and glycolysis, measured as oxygen consumption rate and extracellular acidification rate, respectively [63]. Further analyses reveal downregulated glycolytic enzymes and unaltered activity of the electron transport chain enzymes, suggesting the causal role of glycolysis dysregulation in this CNS pathogenesis [63]. Reduced glycolysis and mitochondrial respiration have also been reported in the zebrafish epilepsy model caused by STXBP1 deficiency [62], representing a simple non-invasive metabolism-based approach that can be used for real-time monitoring epilepsy and drug responses in zebrafish.

Such strategies may also be useful for uncovering novel anti-seizure drugs [110], for instance, identifying vorinostat (a histone deacetylase inhibitor) as a potential anti-seizure compound based on its ability to restore metabolic parameters (e.g., basal respiration, maximal respiration, mitochondrial respiration, proton leaks, and ATP-linked respiration) in both pharmacological (pentylenetetrazol-induced) and genetic (*kcna1*-morpholino-induced knockdown) zebrafish models of epilepsy [64]. The efficacy of vorinostat as an antiepileptic agent has been further validated in mice using behavioral and EEG markers [64]. Metabolic-based small molecule screening has also been successful in the zebrafish model of the Dravet syndrome discussed above, since PK11195 (a compound known to enhance gluconeogenesis) normalizes behavior and restores hypometabolic phenotype of mutant zebrafish, correcting hypoglycemia and reduced rates of mitochondrial respiration and glycolysis [61]. In addition to emphasizing the value of peripheral metabolic biomarkers for

specific CNS disorders, these findings support the use of the energy-producing pathways as novel therapeutic targets for the treatment of brain disorders.

Parkinson's disease. Mitochondrial dysfunctions and oxidative stress are strongly implicated in the Parkinson's disease pathogenesis [111, 112], a highly prevalent and severely debilitating progressive neurodegenerative disorder. Some drugs that directly interfere with mitochondrial function can induce Parkinsonism in both humans and animals [113-115]. Likewise, zebrafish exposed to mitochondrial dehydrogenase inhibitors, such as rotenone, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), 1-methyl-4-phenylpyridinium (MPP^+), 6-hydroxidopamine and paraquat, develop motor deficits, loss of dopaminergic neurons, and γ-synuclein aggregation, providing good pharmacological models of Parkinson's disease [116]. Altered mitochondrial function and redox status in these models are reflected in various biochemical biomarkers that can be reliably measured in whole-body zebrafish samples.

For example, MPTP induces mitochondrial dysfunction in zebrafish, as reflected by decreased activity of mitochondrial complex I and downregulation of mitochondrial proteins parkin, DJ-1, and PINK1 [72]. These proteins are crucial for mitochondrial homeostasis and oxidative stress protection, and their dysfunction is associated with familial cases of Parkinson's disease [117]. Interestingly, melatonin treatment restores both proteins content and complex I activity, as well as normalizes larvae behavior, supporting its therapeutic potential for Parkinson's disease [72]. The whole-larvae mitochondrial bioenergetics is also affected in the paraquat- [71] and MPP^+-induced zebrafish models of Parkinson's disease [70].

Furthermore, the neurotoxin-exposed zebrafish present classical biomarkers of oxidative stress, such as increased lipid peroxidation and decreased glutathione level [73-75], including altered activity of the enzymes participating in the oxidative stress response, such as catalase [74, 118], glutathione-S-transferase [73, 74, 118], and superoxide dismutase [73, 74]. Notably, oxidative stress biomarkers are specifically altered in the intestines and brains of the rotenone-exposed zebrafish [73, 74]. Given some evidence of the causal role of gastrointestinal dysfunction in Parkinson's disease [119, 120], the rotenone-exposed zebrafish may represent an interesting model to study oxidative stress related gut-brain interactions relevant to Parkinson pathogenesis [74].

DISCUSSION

As animal models continue to facilitate studying mechanisms of complex brain disorders and drug discovery, the zebrafish is rapidly becoming a valuable model object in the translational neuroscience and probing a growing number of CNS diseases. These fish share some practical advantages over mammalian models together with conserved biochemical and neurobehavioral features. Overall, like in humans, CNS pathogenesis in zebrafish is associated with the disease-specific biochemical changes, impacting concentrations of lipids, carbohydrates, proteins, and other non-signal molecules, as well as enzymes activities and rates of metabolic reactions. Importantly, the signs of biochemical dysregulation can be detected not only specifically in the brain, but also in peripheral tissues and whole bodies of zebrafish. This, together with the use of elaborated analytic methods (e.g., high performance liquid chromatography-mass spectrometry [HPLC-MS]), facilitates biomarker detection process by making it less time- and labor-consuming. As discussed above, this strategy has been applied to a diverse group of zebrafish models of CNS diseases (e.g., stress-related affective disorders, NPC, epilepsy, Parkinson's disease), where the non-brain biochemical biomarkers are used for three major purposes (Table 3).

Firstly, they can be used for the validation of a particular model, provided that the observed biochemical changes parallel those seen in the clinic. This case is common in the models of CNS diseases with clearly established biochemical profile, where, for example, increased levels of lysine catabolites serve as reliable biomarkers of pyridoxine-dependent epilepsy in zebrafish [67, 68]. Another application is drug screening, where the reversal of the impaired biochemical profile provides a clear indication of drug effectiveness. Several studies, as mentioned above, show sensitivity of zebrafish biochemical biomarkers to pharmacological interventions [59, 61, 65, 73, 106], thus promoting the biomarker-based high-throughput drug screening in zebrafish. Indeed, at least one compound identified in zebrafish by its ability to restore metabolic aberrations in epilepsy, vorinostat, now undergoes clinical trials [64, 121]. Finally, zebrafish may serve as a useful low-cost platform for the development of biomarkers that provide new insights into the pathophysiology of CNS diseases, such as the developed zebrafish 3D maps of lipid distribution in NPC [60] and profiling amino acid dysregulation in the models of B6-responsive epilepsies [65, 66, 69].

PROBLEMS, LIMITATIONS, AND FUTURE RESEARCH

Although focused on peripheral biomarkers, only few studies have actually investigated the samples of peripheral tissues without brain tissue, rather than

using whole-body samples. As such, it is not always clear whether the described biomarkers are actually of peripheral, central, or rather of "systemic" nature. A major advantage of the whole-body approach is that it is fast and does not require tissue dissection, hence, markedly facilitating rapid biomarkers determination. This is particularly relevant for the experiments with small-sized larval and embryonic zebrafish. For example, in the zebrafish model of the Dravet syndrome, the low glucose levels clearly correlate with seizure-like behavior, thus serving as likely biomarkers of drug responsiveness as well [61]. At the same time, because such analyses are performed on the pooled homogenized bodies of zebrafish larvae, it is unclear whether hypoglycemia is due to the increased brain energy demands or peripheral glucose depletion. On the one hand, practically speaking, it is not always necessary to know the exact origin of the biomarker, if it fulfills its purpose. On the other hand, further studies may need to use more specific techniques for tissue collection, revealing the exact origin for each biomarker used.

Studying biomarkers in animal models also has another benefit – the possibility of finding artifacts specific to the animal model(s), rather than to the modeled disease itself. In the present context, this can be attributed to distinct biochemical features of zebrafish (from those seen in mammals), differences in the applied experimental manipulations that may only mimic certain, but not all, etiological factors of 'real' human CNS diseases. For example, in the stress-evoked metabolomic responses, the observed biochemical biomarkers may reflect zebrafish response to specific experimental conditions (e.g., stressors) rather than universal reaction to stress *per se* [52]. Likewise, various oxidative stress biomarkers found in the intestines of zebrafish exposed to rotenone [73, 74] – a compound known to induce oxidative stress – can simply confirm the peripheral activity of the applied neurotoxin, rather than inform us on Parkinson's pathogenesis. To overcome this problem, one strategy can be to test such findings using different animal models, also increasing the range of disease-inducing factors (i.e., combining pharmacological, genetic, and environmental manipulations) and employing a wider range of model organisms, aiming at a greater generality of a model. Translational relevance of the specific disease biomarker in question can then be more reliably established based on how it integrates into the existing complex pathophysiological picture of the modeled disease.

Finally, the zebrafish as a model organism has some other, species-specific limitations (Table 1) that may become critical for CNS disease modeling. For instance, the zebrafish possesses several neuroanatomical features (e.g., the lack of neocortex) that, albeit characteristic of all lower vertebrates [16], can hinder direct translation of zebrafish disease phenotypes into humans [122]. Another example is the lack of midbrain dopaminergic neurons in zebrafish, which is particularly pertinent when modeling Parkinson's disease. In humans, Parkinson's disease is characterized by degeneration of dopaminergic neurons in the substantia nigra and ventral tegmental area situated in the midbrain [123, 124]. However, in the zebrafish models, it is the diencephalic populations that are primarily affected [113, 125, 126]. While some studies propose that dopaminergic neurons in the diencephalon of zebrafish may serve as functional analogs of the human midbrain neuronal populations, this question is debated [38, 127, 128]. Thus, while behavioral and physiological phenotypes observed in zebrafish models of Parkinson's disease resemble those in mammals, the degree to which the findings from zebrafish studies can be extrapolated to humans remains uncertain.

Furthermore, due to additional round of genome duplication that occurred in the teleost fishes millions of years ago, zebrafish have more copies of many orthologous human genes, including those implicated into CNS diseases [129, 130]. This, in turn, may complicate such research in zebrafish, including development of the genetic knock-out models and interpretation of the results of pharmacological studies. Furthermore, while zebrafish is perfectly suitable for administration of water-soluble drugs (through water immersion), some compounds are water insoluble and may necessitate the use of organic solvents or injections, thus increasing the number of factors influencing the experiment [131]. Lastly, being a relatively new model object in neuroscience, zebrafish lack some convenient features of the traditionally utilized rodents, such as the availability of multiple fully characterized inbred strains or behavioral tools [26, 132]. Although many zebrafish behavioral paradigms have been successfully adopted from rodent models [83, 133-135], some complex behaviors (e.g., fine motor coordination or parental care) cannot be assessed in zebrafish experiments despite being highly relevant to many CNS disorders.

CONCLUSION

Brain disorders often have pathological manifestations occurring peripherally or at the whole-organism level, which strongly supports the use of peripheral biomarkers for diagnostic and therapeutic purposes. Zebrafish models are becoming an important tool in the field of translational neuroscience [16, 136], also providing useful models of human CNS pathologies, where reliable biochemical changes can be detected either in samples of peripheral tissues or whole-bodies. Zebrafish models often demonstrate similar pattern of

biochemical dysregulation to those observed in clinical studies, supporting the developing utility of zebrafish to study peripheral biochemical biomarkers of brain disorders, and their value for the mechanistically-driven, target-based discovery of novel therapies.

Contributions. A.V.K. conceived and supervised the study; N.P.I., T.O.K., and S.L.Kh. analyzed the data and discussed findings with input from all authors; N.P.I. and K.A.D. wrote the manuscript; E.V.P. and A.V.K. edited the manuscript.

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