● REVIEW

The metabolome identity: basis for discovery of biomarkers in neurodegeneration

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Abstract

Neurodegenerative disorders are often associated with cellular dysfunction caused by underlying protein-misfolding signalling. Numerous neuropathologies are diagnosed at late stage symptomatic changes which occur in response to these molecular malfunctions and treatment is often too late or restricted only to the slowing of further cell death. Important new strategies to identify early biomarkers with predictive value to intervene with disease progression at stages where cell dysfunction has not progressed irreversibly is of paramount importance. Thus, the identification of these markers presents an essential opportunity to identify and target disease pathways. This review highlights some important metabolic alterations detected in neurodegeneration caused by misfolded prion protein and discusses common toxicity pathways identified across different neurodegenerative diseases. Thus, having established some commonalities between various degenerative conditions, detectable metabolic changes may be of extreme value as an early diagnostic biomarker in disease.

Key Words: *metabolome; neurodegeneration; neuroinflammation; nitric oxide; redox stress; biomarker; misfolded protein; prion disease*

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Introduction

Metabolomics is becoming an essential tool for systemic characterization of metabolites in order to provide a snapshot of the functional and pathophysiological states of an organism and to support disease diagnostic and biomarker discovery. Already detailed information of numerous metabolites found in human biospecimens, such as blood, urine or cerebrospinal fluid is deposited in the Human Metabolome Database (http://www.hmdb.ca/). To generate meaningful data the samples were collected from a large cohort of pathologically-verified patients and aged-matched controls and analyzed by using ultra performance liquid chromatography and gas chromatography coupled to various mass spectrometry platforms. As a result, important disease-relevant markers were identified and include the formation of the 3-hydroxykynurenine and reductions of antioxidant glutathione signaling which are now recognized as important biomarkers of excitotoxicity and oxidative stress in the pathogenesis of neurodegenerative conditions such as Parkinson's or Alzheimer's disease (Lewitt et al., 2013). Cerebrospinal fluid metabolomics, in particular, provided specific advance in the characterisation of disease progression and development of biomarkers. As such, changes in certain cerebrospinal fluid metabolites associated with developing neuronal degeneration from mild cognitive impairment to Alzheimer's disease include biosynthesis and metabolism of lipids, cortisone and certain amino acids, markers of mitochondrial function and energy production as well as urea cycle and bile acid metabolism (Laurens et al., 2015).

In order to understand observed changes in the metabolome, it is vital to monitor the time course of metabolic changes which occur during the pathology of disease to characterise abnormalities at early stages and thus allowing more specific treatment approaches. Utilising the wealth of available disease model systems, the challenge will be to identify early markers of disease. The underlying pathology of many neurodegenerative conditions precedes the appearance of symptoms, sometimes by many years or even decades. The metabolome is a dynamic and sensitive biological system, which is determined by both innate processes and environmental impacts. To an extent, it encodes an organism's health and homeostatic balance. Thus, early or even pre-symptomatic diagnosis could be possible by identifying metabolomics biomarkers of disease, as some studies have demonstrated (Bamji-Stocke et al., 2018; Dong et al., 2018; Yu et al., 2018). **Figure 1** illustrates verified pathways which are either causally or secondarily involved in various neurodegenerative conditions and it remains to be confirmed in which sequence these changes occur in order to identify potential biomarkers for diagnostics. We have performed a PubMed literature search of articles published in the period 2005–2018 on metabolic changes in neuropathologies and specifically highlighted publications illustrating redox signaling with a potential for treatment targets.

Commonalities of Misfolding Protein Signaling

It is important to recognise that research on the human metabolome related to neurodegeneration delivered numerous correlative changes in metabolites with disease progression. Neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease and Huntington's disease are characterised by a progressive decline in neuronal health leading to cell death. Hallmarks of these diseases in-
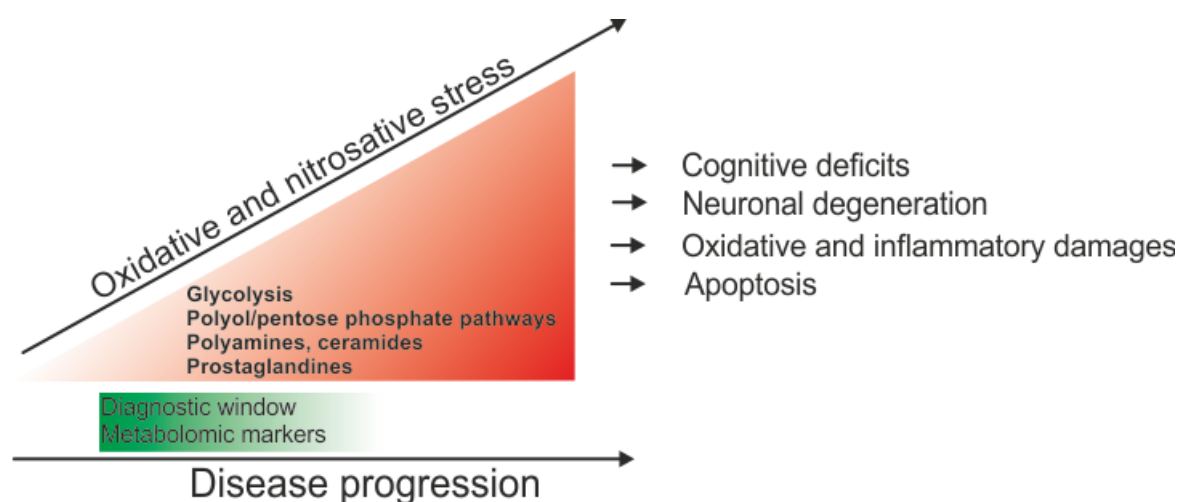
Figure 1 Disease relevant alterations in neuronal metabolism with potential for biomarker development.
As neurodegenerative disease progresses, several metabolic pathways are altered and early detection of these changes could provide support for development of diagnostic biomarkers and treatments. Disease progression advances with increasing redox stress which provides a favourable target for treatment strategies.

clude the accumulation of misfolding proteins and protein aggregation, development of spongiform lesions, gliosis and neuronal loss. The accumulation of misfolded proteins in neurodegenerative diseases across several degenerative conditions which include β-amyloid, α-synuclein, huntingtin or the scrapie form of the prion protein is believed to cause an initial damage leading to cell death signaling. As such, several over-activated pathways show strong commonality across these different diseases and include the unfolded protein response, neuroinflammation and oxidative stress signaling resulting in global metabolic dysfunction. Importantly, the use of animal models allows the use of more detailed analysis strategies. Different animal model systems of disease confirmed metabolic changes observed in human because metabolites tend to be conserved across species, from bacteria to mammals, and thus insights gained from metabolomics studies in model systems can be more readily translated to human data. Importantly, metabolites are easily accessible *in vivo*, allowing the assessment of organismal health in real time, indispensable for medical diagnostics.

As illustrated in our recent metabolomics study, one common factor in the pathology of neurodegeneration is neuroinflammation which substantially contributes to disease progression. In our study we utilised the well characterised neurodegenerative disease caused by misfolded prion protein as a model for neuropathology caused by protein misfolding (Bourgognon et al., 2018). The ubiquitously expressed cellular prion protein (PrP^C) is a copper-binding glycosylphosphatidylinositol anchored protein localised in membrane lipid raft microdomains where it interacts with a variety of proteins involved in regulating synaptic transmission. Prion protein facilitates several neuronal processes, including transmitter release at glutamatergic synapses, is involved in mediating neu-

ronal plasticity by supporting neurite outgrowth and it regulates neuronal Ca^{2+} signaling. Under physiological conditions PrP^C possesses antioxidant activity but during prion disease, it undergoes a profound conformational rearrangement to a β-sheet-rich structure termed scrapie prion (PrP^{Sc}) that is aggregation prone and infectious. PrP^{Sc} is self-propagating and binding of PrP^{Sc} to PrP^C leads to the conformational conversion of PrP^C into an additional copy of PrP^{Sc} (Aguzzi and Calella, 2009). In order to examine the mechanisms that are potentially affected during the development of this protein-misfolding disease, we investigated the metabolic alterations in the cortex and hippocampus of prion-diseased mice. We studied metabolomics changes at a stage of disease when synapse and neuronal loss, astrogliosis and memory deficits are detectable but yet confirmatory signs of the disease are absent.

In analogy to neurodegenerative conditions studied in human, our data revealed significant cortical and hippocampal alterations in glucose metabolism, amino acid utilisation and complex lipid homoeostasis, which potentially exacerbate progression of the disease. Abnormal glucose homeostasis has been described in patients with Alzheimer's disease (An et al., 2018). In late-onset Alzheimer's disease, mitochondrial metabolism is impaired and there is an increase in energy production mediated by glycolysis (Sonntag et al., 2017). These observations are indicative of excess oxidative stress that impairs mitochondrial energy production. Our study revealed major alterations of glucose metabolism and energy production in prion-diseased mice with glycolysis and polyol/pentose phosphate pathways being strongly upregulated.

The glycolytic pathway is directly linked to energy production *via* pyruvate, oxidative phosphorylation and ATP production in the mitochondria. These data could feed into the hypothesis that a deficient energy metabolism

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resulting from defective mitochondrial function and increased oxidative damage may contribute to neuronal dysfunction and degeneration. Classically, elevated levels of NADPH are a cellular response to prevent oxidative stress and maintain the redox balance, as it is the case in the hippocampus of prion-diseased mice which display signs of oxidative stress.

Neuroinflammation and Nitric Oxide Signaling

Augmented nitric oxide signaling is associated with the immune response and plays a key role in regulating inflammatory processes and redox stress in numerous pathologies including neurodegenerative conditions, various forms of vascular dysfunction, sepsis or forms of cancer (Steinert et al., 2010; Nakamura et al., 2013; Tesfai et al., 2017; Wang et al., 2018). It has become increasingly evident that redox modifications play pivotal roles in mediating downstream signaling pathways that regulate normal biological and physiological processes. Nevertheless, their dysfunctional regulation is associated with many pathologies. To act as effective biological signaling molecules, reactive species including the free radical nitric oxide, but also its high and low molecular weight carrier molecules containing S-nitrosothiols (in particular S-nitrosoglutathione) have the ability to induce reversible and functional alterations in the protein activity, interactions and stability. However, these modifications are also recognised as relevant singling pathways in various neurological pathologies such as Alzheimer's disease or Parkinson's disease (Nakamura et al., 2013; Bradley and Steinert, 2016; Nakamura and Lipton, 2017). The redox-based post-translational modification of proteins mainly occurs to the thiol side chain on specific cysteine residues of targeted proteins which can produce a wide variety of chemically distinct alterations that underlie homeostatic control. These diverse biological pathways, affecting neuronal communication, present attractive targets for treatment in neuropathology (Nakamura and Lipton, 2016). An important aspect of nitric oxide signaling in the brain is mediated *via* regulation of vascular activity. Such signaling involves neurovascular coupling and its disturbance is associated with aging and neurodegeneration (Lourenco et al., 2017). However, our data indicate that in neurodegeneration elevated levels of the competitive endogenous inhibitor of nitric oxide synthases, asymmetric dimethylarginine, may modulate the production of reactive oxygen and reactive nitrogen species which has been shown in inflammatory signaling in the lung. Experimental and clinical evidence demonstrates that even small modifications of asymmetric dimethylarginine concentrations significantly change vascular nitric oxide production, vascular tone and systemic vascular resistance (Bode-Boger et al., 2007) which may have a negative impact on neurovascular coupling but simultaneously diminish neurotoxic nitric oxide signaling generated by

the inducible nitric oxide synthase isoform.

Another regulatory compound involved in nitric oxide signaling is L-arginine that also impacts on the metabolism of polyamines, proline, glutamate, creatine and agmatine. It is involved in two major metabolic pathways, the nitric oxide synthases pathway where L-arginine is converted into nitric oxide and L-citrulline and the arginase pathway. Both compounds affect the vascular system as endogenous antiatherogenic molecules that protect the endothelium and modulate vasodilatation. Together they can contribute in the brain indirectly and directly to the regulation of neuronal function, neurotransmitter homeostasis involved in learning and memory, synaptic plasticity and neuroprotection (Garthwaite, 2016).

Most of the L-arginine-related metabolites are upregulated in prion-infected brains confirming a strong increase in nitric oxide signaling. This closely relates to the enhanced neuroinflammatory activity reported in many other neurodegenerative conditions, with a strong activation of microglia and subsequent upregulation of inducible nitric oxide synthase expression leading to nitric oxide-induced neurotoxicity. This signaling enhances nitrergic stress and can lead to unbalanced formation of nitric oxide-mediated post-translational modifications with resulting neuronal and synaptic dysfunction (Calabrese et al., 2009; Steinert et al., 2010).

Altered Polyamine Metabolism

As above signaling impacts on polyamine metabolism, it is conceivable to suggest that spermidine and spermine might be affected in disease. Both compounds are present in neurons and glia and protect mitochondria against mitochondrial permeability transition acting as potent antiapoptotic factors. Spermine acts as a free radical scavenger against reactive oxygen to prevent oxidation of mitochondrial transmembrane potential-related proteins like glutathione and other proteins containing sulfhydryl groups. However, some reports favour a pro-apoptotic role of polyamines with the oxidation of polyamines leading a marked intracellular accumulation of amine linked to elevated hydrogen peroxide levels. Levels of polyamines are changed in Alzheimer's disease and Parkinson's disease possibly due to interactions with the L-arginine signaling cascade. A toxic elevation of polyamines in neurodegenerative conditions could translate into an excessive activation of NMDA receptors together with an aberrant activation of apoptosis. There is a growing body of evidence suggesting that ceramide can facilitate permeabilisation of the mitochondrial membrane (Taha et al., 2006) and drive cell death. Oxidative stress activates sphingomyelinases which in turn hydrolyzes sphingomyelin, yielding phosphorylcholine and ceramide. The latter is upregulated in prion diseased mice. The augmentation in ceramide levels leads to apoptosis and further neuronal degeneration. In this context, ceramide levels have been reported to be ele-

vated in brains from Alzheimer's disease patients making this pathway a strong potential contributor to neurodegeneration.

The available data on metabolomics in degenerative diseases offer an enormous potential to develop treatment strategies in human but also illustrate how the immense complexity and interactions of described pathways can complicate this field of research. Thus, the challenge remains to systematically produce a classification of metabolic pathways and their alterations at early pre-symptomatic pathology stages to allow meaningful interpretations and biomarker development.

Conclusions

As illustrated above, multiple pathways are altered during the pathogenesis of neurodegenerative diseases and advances in techniques have stimulated a great interest in the application of metabolomics to explore neuropathologies. Since metabolic pathways are largely conserved between species it is a key tool for translating results from cellular and animal models to human. Applications of metabolomics can reveal novel candidate metabolites in different biological samples such as cerebrospinal fluid, plasma, urine and serum by using multiple mass spectrometry-based platforms in human or animal models of neurodegeneration (Ibanez et al., 2015). As reported in our study (Bourgognon et al., 2018), analysis of metabolites in brains of a prion-diseased mice revealed numerous disease-relevant alterations in neuronal metabolism, some of which have been reported in other neurodegenerative conditions in human. Thus, the use of mammalian model systems for metabolomics studies not only contributes for better understanding of complex mechanisms of diseases development but also supports the discovery of potential biomarkers for diagnosis.

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