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Microdissection and transcriptional profiling
A window into the pathobiology of preclinical prion disease

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Prion diseases share common features on a sub-cellular level with many neurodegenerative diseases including Alzheimer disease; the most prevalent neurodegenerative disease world-wide. The most obvious similarity is the accumulation of misfolded forms of the host proteins which forms aggregates in the brains of patients. Remarkably, one of the earliest pathological changes detected in degenerating brain tissue, well before clinical symptoms are observed, is synaptic dysfunction and loss. This pathology was recently shown to be reversible in early stages of mouse prion disease suggesting that synaptic regeneration and reestablishment of neuronal function is possible. Determination of the molecular events that underlie synapse degeneration and how this eventually results in neuronal loss is therefore a research priority that may contribute to the search for new therapeutic interventions for neurodegenerative disorders. Functional genomic studies using unbiased whole genome expression analyses represent one method that can provide insights into these perplexing processes. However, transcriptional profiles from brain tissues are representative of a heterogeneous mixture of cell types that effectively mask the expression of low abundance transcripts, or molecular changes that occur only in a small population of affected neurons. One method that was recently applied to address these challenges was laser capture microdissection which was used to effectively isolate the CA1 neuronal rich region of the hippocampus prior to RNA extraction. Profiling of both mRNAs and microRNAs revealed previously unidentified neuronal-specific genes and expression signatures that are relevant to understanding the pathophysiological processes involved in preclinical stages of prion disease. In this review we will highlight these molecular signatures and discuss their implications with respect to prion-induced neurodegeneration.

Introduction

Prion diseases are rare and uniquely infectious neurodegenerative diseases that share many common sub-cellular features with more prevalent neurodegenerative diseases including Alzheimer disease. The most obvious similarity is the strong association with the accumulation of misfolded, aggregated and insoluble forms of normal host proteins in the brains of patients followed by the eventual and irreversible loss of neurons. Remarkably, these neurodegenerative diseases also appear to share one of the earliest pathophysiological events of neurodegeneration, namely synaptic dysfunction and loss.[1,2] Synapse degeneration begins well before clinical signs are conspicuous and it was recently shown that this early damage is reversible in prion infected mice by conditional knockdown of the normal cellular form of the prion protein.[3] Intervention in the process that leads to synaptic toxicity is therefore a promising target for the development of novel therapeutic approaches, not only for prion disease but also for a wide range of neurodegenerative disorders. Determination of the exact molecular events that underlie synapse degeneration and how this eventually results in neuronal loss is therefore a research priority. Functional genomics using unbiased whole genome expression analyses are one method that can be used to provide insights into these perplexing processes.

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Synaptic Degeneration

The loss of synapses has been a recognized feature of neurodegenerative disease pathology for many decades. This process is not uniform throughout the infected brain and may reflect the selective vulnerability of particular neuronal populations based on their intrinsic (genetic components) or extrinsic (local microenvironments) properties. For example, the hippocampus and cortical regions of both humans and animals show the most consistent decrease in synaptic densities in response to infection with various prion strains as quantified by either electron microscopy[4] or by immunohistochemical staining for synaptic markers.[5,6] More specifically, prion patients show abnormalities in synapse organization and morphology, a reduction in the amount of staining of the synaptic protein marker synaptophysin and degeneration of pre-synaptic terminals.[4,7] In addition to synapse loss, reduction in dendritic spine number is especially noticeable in the stratum radiatum of region 1 of the *Cornu Ammonis* layer (CA1) of the hippocampus in the majority of murine prion models. Within this region, the degeneration appears to involve both pre-synaptic and post-synaptic components[2] while in the ME7

model it has been reported that degeneration first occurs in the pre-synaptic boutons from CA3 axons terminating on the spines of CA1 neurons.[2,4]

Recent biochemical data has revealed a program of events that defines the loss of synapses. Initially, proteins implicated in the pre-synaptic terminal compartment, particularly those involved in synaptic vesicles, were decreased.[8] Electron microscopy studies showed that the degenerating pre-synaptic boutons were found to be progressively enveloped within the post-synaptic density of dendritic spines.[4] Concurrently, morphological changes including swelling and hypertrophy in apparently intact pre-synaptic terminals have also been reported.[9] Importantly, during early disease, the loss of dendrites and the decrease in the number of intact synapses appears to be disproportional to the number of neuronal cell bodies that are lost.[10] Collectively, these studies suggest that affected neurons, although phenotypically altered, remain viable for significant periods of time in the brains of affected individuals, even maintaining a capacity for synaptic repair.[3,11] Since the functional status of cells is determined to a large extent by their genomic activity in terms of de novo transcription, this opens a window for genetic analysis to identify the temporal molecular changes that occurs during these very early stages of disease.

Transcriptional Profiling of Degenerating Neurons

Numerous reports of gene expression changes from prion-infected whole mouse brain tissue have been published over the last decade (reviewed in ref. 12). Similar transcriptional profiles have been detected within other animal models, such as BSE-infected cattle (reviewed in ref. 12) as well as in human sporadic Creutzfeldt-Jakob disease patients.[13] Although further discussion of these studies is beyond the scope of this review, the molecular responses detected in these samples mirror that of the mouse prion models and further supports the utilization of the mouse models for studying the neurodegenerative process that is also observed in larger animals and human patients afflicted with prion disease. Typically, such high-throughput studies are very challenging to interpret because of the cellular and functional complexity of the brain. Brain tissue is made up of a myriad of neuronal cell types that work together in intricate cellular networks. Adding to this complexity is the multitude of supporting cells such as astrocytes, microglia and oligodendrocytes that outnumber neurons by at least 10:1, even in the healthy brain. Therefore, whole brain mRNA profiles are representative of a formidably heterogeneous mixture of cell types. Expression signatures of low abundance transcripts as well as subtle temporal changes, or those restricted to small populations of affected neurons, are ultimately masked when analyzing whole brain tissue. Isolating cell types prior to genomic analysis poses steep technological challenges that have been met with the implementation of various methodologies. In our own work we have applied laser capture microdissection (LCM) technology to specifically "cut-out" degenerating neurons from frozen brain tissue sections.[14,15] To our knowledge, this is the first application of such cell-type specific gene expression profiling methodology in prion disease. A number of other methodologies exist for cell enrichment that could be similarly utilized, including translating ribosome affinity purification,[16,17] fluorescence activated cell sorting,[18,19] immuno-panning[19] and manual sorting;[20] the details of which are provided in the corresponding references.

We chose to isolate CA1 neurons because these cells show significant reduction in dendritic spine numbers at 35–45% of the incubation period in intraperitoneally inoculated mice. Furthermore, these neurons show minimal death detected prior to the onset of clinical disease, a pathology that is observed after the completion of more than 70% of the disease process.[10] On a practical level, neurons within the CA1 region also represent a relatively homogeneous population of densely packed cell bodies for isolation using LCM.[21] In support of this cell-type specificity, we found that the cell bodies of activated astrocytes and microglia, major players in the innate immune response of the brain, do not significantly infiltrate the CA1 neuronal layer until clinical stages of disease. Accordingly, mRNAs coding for microglial and astrocyte genetic markers such as glial fibrillary acidic protein (GFAP), allograft inflammatory factor 1 (AIF1) and cytokines/chemokines produced upon activation were not detectable by microarray analysis of microdissected CA1 preparations until the incubation period reached at least 60–70% of the disease course. Conversely, neuronal expressed genes such as the neurofilament medium and light polypeptides plus synaptosomal-associated protein 25 (SNAP25) were highly enriched in the sampled tissue at all stages of disease. Collectively, this data clearly suggest that the LCM-based approach was highly effective at sampling neuronal specific cell populations, allowing us to explore molecular responses induced specifically within neurons during prion infection.

Overall, our gene expression profiles from microdissected neurons revealed two major temporal molecular phases. First, a dominant cluster of altered transcripts was evident during preclinical disease. Subsequently, these genes either returned to basal expression levels or alternatively, underwent a direct reversal in expression profile during clinical disease. We also identified a second cluster of altered transcripts concomitant with the onset of clinical signs along with the steady increase in inflammation related genes presumably expressed by infiltrating microglia and activated astrocytes. Although the dysregulation of numerous neuronal expressed genes is also evident at clinical disease, the major features of this molecular signature are reminiscent of transcriptional analyses of mixed cell-types. In particular, the genes involved in the chronic inflammatory response that accompanies neurodegeneration are clearly discernible.

Preclinical CA1 Transcriptional Profiles

To investigate causal relationships in prion infection it is essential to determine what the earliest disease related processes are and whether their progression can be correlated with the onset of clinical symptoms. Understanding transcriptional changes prior to clinical disease and linking those changes to the observed pathobiology are a major focus of our laboratory. In

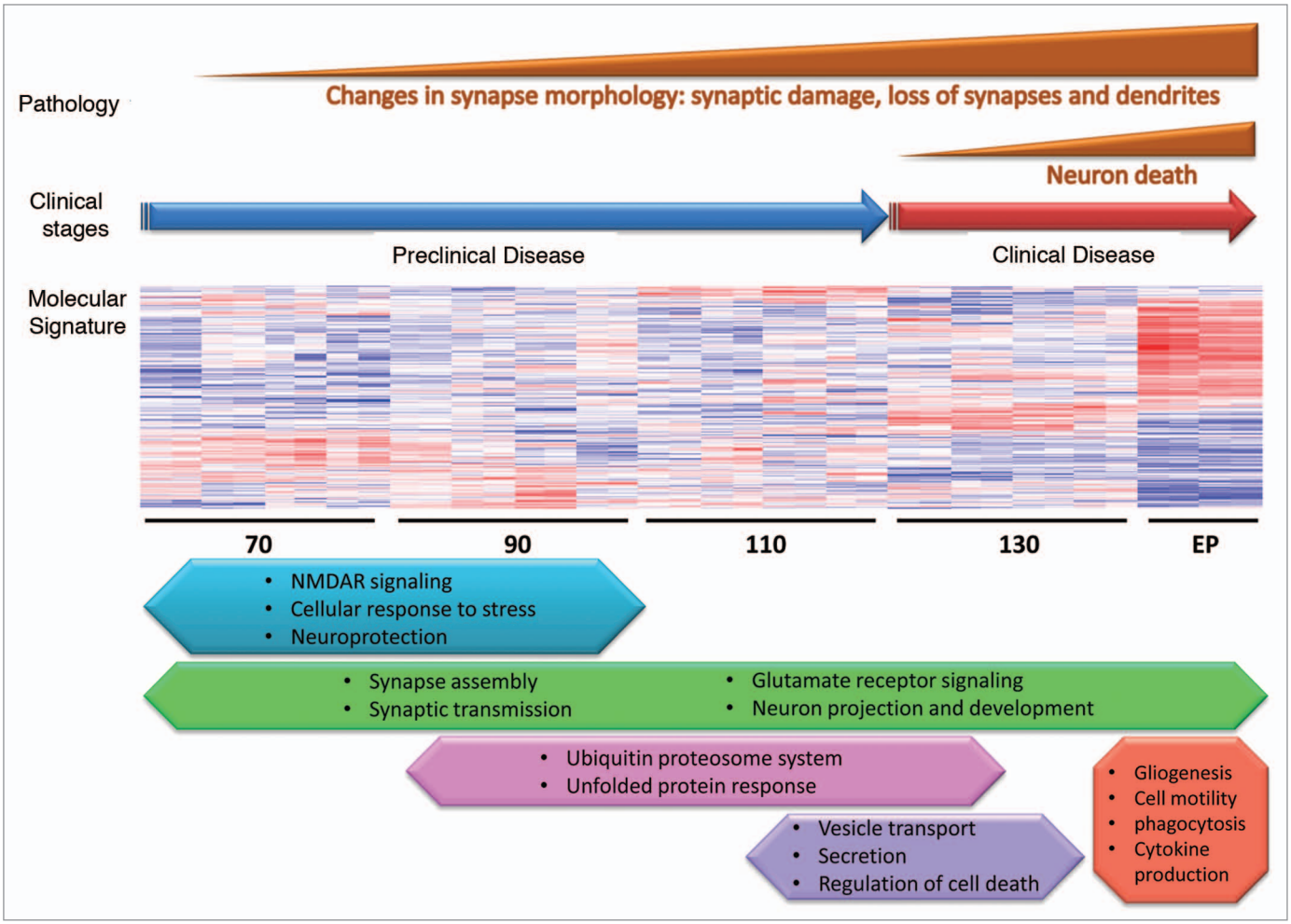

Figure 1. A schematic overview of the molecular networks deregulated during prion infection. The molecular signature presented here is based on data obtained from neuronal-specific transcriptional profiling of the CA1 hippocampal region.[15] In this animal model, approximately 70% of the disease course represents the preclinical stage (blue arrow). These animals experience changes in synapse morphology beginning at 70 DPI which leads to synaptic damage, loss of synapses and dendrites as disease progresses. This pathology accumulates over the entire preclinical stage of disease which perpetuates death of neurons observed during the clinical stage (red arrow). Mapping of gene expression profiles onto this pathological underpinning revealed the deregulation of numerous pathways at different times in disease. Overall representation of temporally deregulated gene expression profiles is depicted by the heat map where red lines represent upregulated genes while blue lines represent downregulated genes in prion infected samples as compared with controls. Deregulation in processes involved in organization and regulation of synaptic function (green and blue banners), neuronal survival pathways (blue banner) and stress associated protein kinase pathways (purple banner) were deregulated throughout prion disease. Immune related processes (red banner) are primarily observed at clinical disease. The positions of the molecular signature banners relative to the timeline of disease approximate the temporal footprint at which these processes show deregulation at the molecular level. EP stands for endpoint and represents the approximate 160 DPI in this animal model.

terms of gene ontology we found prevalent preclinical upregulation of processes associated with cell survival, positive regulation of transcription, stress activated protein kinase pathway, ubiquitin-protein ligase activity and vesicle formation. Downregulated processes were strongly associated with specific neuronal functions including nerve transmission, synaptic components and the postsynaptic density, glutamate receptor activity, glutamate metabolism, γ-aminobutyric acid ($GABA_A$) receptor activity, Ca^{2+} signaling, cytoskeletal organization and synaptic vesicle transport. Although we cannot describe all these alterations in detail, we discuss some of these processes below and comment on how they may underpin pathobiological changes in hippocampal CA1 neurons during prion disease (summarized in **Fig. 1**).

Organization and regulation of synaptic function

In line with the disease-associated pathology a striking downregulation of genes that encode synaptic proteins is apparent from the earliest stages of disease; approximately 35% through the incubation period and onwards. These include genes specifically involved in synapse formation and/or maintenance, such as synaptophysin (SYP), synaptotagmin (SYT1), α-synuclein (SNCA) and SNAP25.[22-25] Many of these genes are also involved in neuronal projection and dendrite development, therefore, modifying general dendrite physiology. This molecular signature

is consistent with the reduction in dendrites that is reported as the earliest detectable pathological change in neurons during prion disease. Interestingly, a number of genes associated with the cytoskeleton and post-synaptic density scaffolds are upregulated pre-clinically, presumably to institute morphological changes such as synapse and dendrite remodelling and loss. By later stages of disease (≥ 65% of the incubation period) these genes are downregulated in accordance with the uniform decrease of genes specifically related to synapse structure and function. Similarly, perturbations in synaptic transmission are implicated throughout disease progression, from 35% of the incubation period and beyond. These include receptors and ion channels such as glutamate receptors and their interacting $GABA_A$ receptors. In addition, phospholipase enzymes appear to be dysregulated and we identified a number of downregulated ryanodine receptors that are located on dendrites and synaptic spines.[26] Activity or dysfunction of these receptors can result in Ca^{2+} release and accordingly we see the alteration in expression of genes involved in calcium ion binding. Although some of these changes have been reported in whole brain analysis,[27-30] the study of microdissected neurons substantially expanded the list of genes to include less abundant transcripts as well as numerous genes of which many have unknown function with respect to neurons or the neurodegeneration process.

Neuronal survival pathways

One of our most striking finding was at the earliest time points analyzed, approximately 35% through the incubation period, when we saw the induction of genes involved in cell survival and viability. More specifically, transcription factors responsible for governing a neuroprotective response was strongly induced during this preclinical time period.[15] Comparing our early gene expression alterations with other molecular signatures reported in the literature revealed the presence of a molecular signature reminiscent of that induced by synaptic N-methyl-D-aspartate receptor (NMDAR) stimulation in primary mouse hippocampal neurons.[31,32] In fact, our preclinical signature included at least 60% of the genes that was reported by Zhang and colleagues[32] to be deregulated by synaptic NMDAR stimulation that acts primarily through nuclear Ca^{2+} signaling and transcription.

NMDAR is an ionotropic glutamate receptor that is primarily involved in synaptic formation and memory function. Interestingly, this receptor is found at both synaptic and extrasynaptic locations on neurons although its stimulation at these two locations could not be more diverse. Evidence suggests that synaptic NMDAR stimulation leads to the activation of a neuroprotective response mediated in part by the transcription factor cAMP response element binding protein (CREB).[33,34] Indeed, we found an abundance of phosphorylated CREB, the active form of the protein, at preclinical disease within the prion-diseased CA1 region while this difference was not detected at clinical disease. At least 35 CREB-regulated genes were also upregulated during preclinical prion disease according to our microarray data. Further to the CREB-mediated pathways, the relative abundance of the NMDAR subunits is suggestive of either neuronal survival, if there are more NR2A subunits or apoptosis if NR2B subunits predominate.[35] Within the CA1 hippocampal neurons we saw an enhancement of NR2A transcripts during preclinical disease, which we deemed to be additional evidence for the induction of a neuroprotective response.

On the other hand, stimulation of NMDARs located extrasynaptically is strongly implicated in the activation of an alternate pathway, omitting CREB activation, which results in cellular death (reviewed in ref. 36). Although extrasynaptic NMDAR stimulation has been previously shown to induce some pro-death related genes, these pathways were not readily detected within our microdissected neurons at any stage of prion disease, even at clinical stages. However, we did detect a small number of individual genes that were previously implicated in neuronal death, such as FOXO1. FOXO1 belongs to the forkhead family of transcription factors and is known to be involved in response to various stimuli including oxidative and endoplasmic reticulum stress and mediates the elimination of damaged neurons by inducing apoptosis.[37]

Interestingly NMDAR signaling has been implicated in a number of previous studies related to the function of the prion protein itself and prion disease. NMDAR signaling in PrP^C-null mice was altered as a result of $GABA_A$ receptor-mediated fast inhibition[38] and increased excitability of dentate gyrus hippocampal neurons from slice culture.[39] Recently, PrP^C-null mice showed increased NMDAR activity causing enhanced excitability states of neurons leading to glutamate excitotoxicity in both in vitro and in vivo models.[40] Collectively, this data proposes that PrP^C is involved in mediating a neuroprotective state by inhibiting NMDAR overstimulation. Hyperexcitation of NMDARs is strongly implicated as a general mechanism that contributes to neurodegeneration not only in prion disease but in many other neurodegenerative conditions including Alzheimer disease. It is postulated that the initial increase in synaptic activation of NMDARs by glutamate is succeeded by synaptic NMDAR desensitization, followed by receptor internalization and activation of extrasynaptic NMDARs and glutamate receptors.[41] This results in the progressive dampening of postsynaptic Ca^{2+} influx and the activation of pathways specific to long-term depression that lead to dendritic spine loss. Over time the resultant loss of synapses presumably reaches a threshold, heralding the onset of cognitive changes and progressive clinical disease.

Stress activated protein kinase pathways

Accompanying the dysregulation of pathways stemming from NMDAR stimulation we also observed alterations in the expression of genes involved in endoplasmic reticulum (ER) associated stress pathways. These include genes such as inositol 1,4,5-trisphosphatereceptors and ryanodine receptors that mediate Ca^{2+} signaling within the ER and mitochondria. Disturbances in ER Ca^{2+} homeostasis and signaling or the accumulation of misfolded proteins can lead to a stress response, both of which are observed in prion disease. ER stress is often accompanied by the activation of the unfolded protein response that employs multiple strategies to return the ER to homeostasis (reviewed in ref. 42). These strategies include the production of chaperones, decreased translation which limits the overall protein load within the cell, upregulation of transcription of ER-resident proteins and

folding assistants as well as factors that facilitate ER-associated degradation (reviewed in ref. 42). Ultimately, if these mechanisms do not restore homeostasis the cell can initiate apoptosis. A number of genes involved in these homeostatic mechanisms were dysregulated in microdissected CA1 neurons including significant induction of numerous ubiquitin ligases and ubiquitin conjugating enzymes that are involved in the ubiquitin proteasome system, enzymes involved in glycosylation plus translational initiation factors that inhibit translation (i.e., EIF2AK1 and EIF2AK3). We also saw upregulation of PUMA, a Bcl-2 homolog 3 known to be involved in endoplasmic stress-induced apoptosis.[43] Few of these genes have been identified in analyses of whole brain tissues during prion disease, however, one study that used macrodissected mouse hippocampal tissue detected enough of these molecules to recognize the induction of some protein trafficking and degradation machinery components.[44] Identification of these genes is significant since recent evidence suggests that the UPR system is chronically overactivated in prion disease leading to long-term translational inhibition that may ultimately cause the decline of critical neuroprotective proteins and the onset of clinical disease.[45] This pathway was also targeted for inhibition and, as a result, was found to ameliorate disease related pathology in the brain and prolong survival in an animal model.[45] Furthermore, oral administration of a pharmacological drug that targets EIF2AK3 rescued infected mice from disease progression.[11]

Preclinical CA1 MicroRNA Profiles

An important posttranscriptional regulatory mechanism that has recently emerged is mediated by short, non-coding RNAs termed microRNAs (miRNAs). These ~22 nucleotide long RNA species have been identified in numerous biological processes holding crucial roles in neuronal differentiation, development, plasticity and survival (reviewed in refs. 46 and 47). In fact, loss of miRNA expression in the brain by inactivation of miRNA processing mediators leads to neurodegeneration in numerous animal models.[48-51] Evidence linking the deregulation of specific miRNAs to the neurodegenerative processes are becoming increasingly apparent[52,53] which further exemplify the important role miRNAs contribute to neurodegenerative diseases. Very little is known about the role of miRNAs in prion associated neurodegeneration therefore justifying the further use of our LCM samples in order to profile miRNAs from CA1 neurons. Interestingly, the overall miRNA expression patterns correlate strongly with that of the mRNA transcriptome in that we also observed two major temporal-specific miRNA clusters; one cluster induced during preclinical and the other during clinical disease.[15]

Approximately 20% of the observed miRNA deregulation was at preclinical stages of prion disease. Generally, little is known about the functions of many miRNAs although a significant number of these deregulated miRNAs are highly enriched in neurons suggesting a neuronal specific function. Indeed, using a bioinformatics approach to analyze the predicted targets of these miRNAs revealed that the targets are highly enriched in biological processes associated with neuronal function, where synaptic organization was the most represented. Therefore, we hypothesize that preclinical dysregulation of miRNAs contributes to synaptic reorganization. MiRNAs are highly suitable regulators at neuronal synapses as they can be sequestered locally at the synapses within P bodies along with their mRNA targets. This capability allows for rapid miRNA:mRNA dissociation in response to synaptic stimuli.[54] Logistically, the production of new transcripts from the nucleus requires additional time for processing, translation and subsequent trafficking of these newly made proteins to the distal sites located within a synapse. The analysis of these distant synaptic sites for mRNA and miRNA dynamics during prion disease would provide valuable information pertaining to the molecular changes taking place at these sites. However, we assessed for nuclear transcriptional dynamics assuming that nuclear transcriptomic changes will reflect which genes and miRNAs are required for replacement and mediation of active synaptic functions.

Several miRNAs dysregulated preclinically have well reported functions within the neuronal context. For example, miR-124a-3p is highly enriched in neurons and can be considered a neuronal-specific marker. MiR-124a-3p promotes adult neurogenesis in vivo[55] and enhanced expression of this miRNA has been shown to promote increased neurite outgrowth.[56] Furthermore, this miRNA also plays a role in preventing apoptosis in hippocampal and retinal neurons during embryonic development.[57] MiR-124a-3p was induced during preclinical prion disease, followed by a steady decline during clinical manifestation. Therefore, the involvement of miR-124a-3p in our preclinical miRNA cluster supports the contribution of this group of miRNAs to the induction of a neuroprotective mechanism identified with the mRNA expression profiles.

Other miRNAs sharing this expression pattern, where preclinical upregulation was followed by decline at clinical stages, were miR-132–3p, miR-29a-3p, miR-16–5p, miR-26a-5p, and miR-140–5p. Of these, miR-132–3p is well-known as a neuroprotective miRNA that is induced by the activation of pCREB,[58] a protein we validated to be present within clinical disease. MiR-26a-5p has recently been identified to mediate dendrite outgrowth,[59] a morphological modification known to mediate neuronal connectivity and function.[60] In turn, miR-29a-3p can affect the shape of dendritic spines by changing mushroom-shaped spines on hippocampal neurons, a phenotype known to mediate strong connectivity between neurons, to filipodia-like outgrowths which is a projection morphology that does not allow for neuronal communication.[61] MiR-29a-3p may therefore function in synaptic and dendritic remodelling as part of a homeostatic mechanism to counterbalance excessive positive cues to stimuli at the synapse[61] perhaps mediated through the over activation of NMDARs. Although the remaining miRNAs, miR-16-5p and miR-140-5p, have poorly characterized neuronal functions to date, their expression within neurons have been confirmed.[15] Interestingly, diminished expression of several of these miRNAs, including miR-29a-3p has also been reported as a general feature of Alzheimer disease representing the likelihood of significant overlap between miRNA functions spanning various neurodegenerative conditions.[62,63]

Differences from Previous Studies in the Functional Processes Identified from LCM Dissected CA1 Neurons

As noted, some of the most prominent incipient prion-related changes from whole tissue analyses (i.e., inflammation, cell adhesion, cell proliferation, energy metabolism, pattern recognition receptors and leukocyte extravasation)[27-30,64-66] were largely absent from the microdissected neuronal material or relegated to the later time-points when disease coincided with the significant infiltration of immune cells into the dissection field. This means that we can start to differentiate between temporal changes in neurons and the interplay with other cells that make up the brain tissue milieu. For example, although the majority of inflammatory genes are not expressed in the neuronal population, a minority of genes with immunological functions begin a sequential overexpression during pre-clinical disease (i.e., ICAM1, VCAM1, FYB, or S100A4) while some genes show a downregulated trend (i.e., IRAK1 or CDC42). The adhesion molecules ICAM1 and VCAM1 are typically expressed on endothelial cells and cells of the immune system, including microglia and astrocytes.[67] In this case, microdissection allows us to track the temporal expression of these adhesion molecules well before immune activation markers are detected, such as cytokines and chemokines. We also noted the increased expression of miR-146a-5p during clinical disease, which is not surprising as this miRNA is known to be expressed from activated microglia.[68] In fact, approximately 77% of the deregulated miRNAs within the CA1 region during clinical disease are known to be deregulated upon astrocyte activation.[69] Clearly, microdissection provides new inroads into the understanding of neuronal-glial communications.

Similarly, we did not observe preclinical alteration in genes involved in endosomal and lysosomal compartments or the autophagic system in LCM neuron enriched samples. Genes related to autophagy have been identified as dysregulated in a number of -omic analyses of brain tissues from prion infected mice.[27,30,45,66] We saw no change in the mRNA levels of the autophagy marker genes BECN1, ATG5, and MAP1LC3B (LC3-II) and upregulation of lysomal compartments was only detected during late clinical phases of disease. This implies that activation of endosomal/lysosomal pathways takes place either in terminal diseased neurons or in infiltrating glia cells.

Although comparing all the biological processes and pathways from our study with those highlighted in previous work is beyond the scope of this review, we wish to mention another example of how such comparisons can be used to interpret inter-cell communication. We noted that the global stimulation of the androgen biosynthesis pathway was a general feature of multiple prion disease mouse models implying that altered levels of neurosteroids may play a role in pathogenesis.[30] However, we did not see evidence of the upregulation of this pathway in our gene expression data although we did observe multiple neurosteroid-induced signaling pathways, such as the aldesterone and corticotropin-releasing hormone signaling pathways, to be significantly deregulated. This comparison suggests that CA1 neurons are not likely to carry out syntheses of these neurosteroids themselves but the genetic effect of their induction is measurable.

Of note, global transcriptional profiling has been performed on numerous prion mouse models where the mouse genetic background, prion strain and route of inoculation varied. Although the specific characteristics of these animal models give way to different inoculation times and dissemination of pathology within the infected brain, all of these animals experience the classic prion pathology and succumb to the disease. Furthermore, a common molecular profile has been observed within all of these animal models, in line with the pathological progression. Therefore, although the disease course may vary within these animal models, the accumulating infectious agent that inevitably stimulates disease progression by affecting neuronal function ultimately results in evoking the same molecular responses within these animals.[27,28,30] It would be most interesting to determine which neuronal subtypes are more resilient to the advancement of the infectious agent; a molecular response best captured during preclinical disease by isolating the candidate neuronal population using a system such as the LCM. To date, only the CA1 hippocampal region has been studied in such a manner within prion disease[15] and further study of different neuronal populations within the mouse brain will help unravel neuronal specific vs. disease specific molecular responses.

Conclusions

These preliminary data only hint at the tremendous amount of information that can be provided by disentangling the role of individual cell types over time during the neural degeneration process. Determining timed dysregulation of molecules (i.e., gene expression, microRNA expression, protein expression or protein modification) in a small number of carefully isolated cells is indeed challenging, and the methods required to circumvent this challenge are at the cutting edge of technology. However, given the size and complexity of the mammalian genome, and the interplay between different cell-types within a tissue as complicated as the brain, we believe that it is essential to extend the power of high-throughput analysis to understanding disease processes in single cells or populations of similarly infected cells. In doing so, we will begin to unravel the mechanisms of disease and response to stress and damage in individual cells. We will also begin to understand the dynamic changes that occur during disease, including how cells communicate with each other to maintain homeostasis under stress, why some cells are more vulnerable than others to specific harmful stimuli, and exactly what and when are the critical points that lead to the terminal, unrecoverable damage of cellular networks. Answers to these questions should allow the formulation of targeted therapeutic approaches to combat complex diseases such as neurodegeneration for which the correct timing and target cell types for application of drugs is critical.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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