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Gene expression changes underlying cortical pathology: clues to understanding neurological disability in multiple sclerosis

Ranjan Dutta, PhD
Department of Neurosciences, Lerner Research Institute, Cleveland Clinic Cleveland, Ohio-44195

Abstract

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system with an unknown etiology. The clinical disease course is variable, with the majority of patients experiencing reversible episodes of neurological disability in the third or fourth decade of life, eventually followed by a state of irreversible progression. Continuous axonal and neuronal loss is thought to be the major cause of this progression. Over the last decade, extensive research has targeted the grey matter and its role in MS pathogenesis. While pathological and imaging studies have begun to reveal important clues about the role of cortical pathology, gene expression studies in MS cortex are still emerging. Microarray-based comparative gene expression profiling provides a snapshot of genes underlying a particular condition and has been performed using brain tissues from patients with progressive MS. In this review, we summarize existing data from gene expression changes in cortical tissues from MS brains and how they may provide clues to the pathogenesis.

Keywords

MS; microarray; neuron; cortex; demyelination

Introduction to cortical pathology in multiple sclerosis

Multiple Sclerosis (MS) is the major cause of non-traumatic neurological disability in young adults in North America and Europe, affecting over 2.5 million individuals worldwide[1,2]. MS is a complex disease whose cause includes a genetic predisposition for susceptibility and environmental influences. Although historically considered a white matter disease, recent advancements in MS research have provided evidence of significant cortical involvement in the pathogenesis with the cortex even having higher lesion load than white matter[1,3,4,5]. Overall cortical lesion burden might affect disability progression as it positively correlates with disease duration, higher EDSS scores and increased cortical atrophy.. Demyelinating cortical lesions are found predominantly in the frontal and temporal cortex, but with reports of demyelination also in the thalamus, basal ganglia, hypothalamus, hippocampus, cerebellum, and grey matter of the spinal cord[3,6]. Demyelination in the cortex is usually associated with the progressive phase of the disease, correlating with the degree of clinical disability[3,5]. Based on the pattern of demyelination, three main types of cortical lesions have been described[7]. Type I or leukocortical lesions are areas of demyelination that contiguously include both cerebral cortex and subcortical white matter. These are informative lesions to

Contact: Ranjan Dutta, Ph.D, Department of Neurosciences, Lerner Research Institute, Cleveland Clinic, Cleveland, Ohio-44195, Ph: 216-445-2671, Fax: 216-444-7927, duttar@ccf.org.

Conflict of Interest
None

study because the white and grey matter portions are of similar ages, making any differences detected due to the environment in which demyelination is occurring. Type II cortical lesions are small perivascular areas of demyelination and do not contribute significantly to cortical lesion load. Type III, or subpial lesions, are bands of demyelination that extend from the pial surface, stopping at cortical layer 3 or 4 and often occupying several gyri. These are the most common grey matter lesions in MS brains[1]. Some Type III lesions may traverse the entire width of the cortex and have been referred to as Type IV cortical lesions[8].

Large scale gene expression profiling provides an unbiased search for candidate genes and pathways altered in MS. Gene expression profiling studies to date have been predicated on the traditional model of MS as a disease where the peripheral immune system targets the white matter. Studies have compared both peripheral blood mononuclear cells and immune cell subsets from MS patients, focusing on patients with relapsing-remitting disease[9,10]. The majority of autopsy studies have looked at the white matter in MS brains. Recent thinking in MS, however, has been directed towards determining the cause of neurological dysfunction in late stage progressive MS[11], where systemic immune responses may be less important and current anti-inflammatory drugs show little benefit. It is in this progressive phase that cortical pathology may be more associated with neurological dysfunction. Examining the genetic substrate of cortical pathology in MS brains should therefore provide a unique opportunity to determine the cause of this neurological dysfunction. Gene expression changes in cortical tissues from MS patients are emerging and Table 1 summarizes major findings of these studies to date.

Gene expression studies in normal appearing cortex

Existing studies looking at MS normal appearing white matter (NAWM) have shown that there are pathological and gene changes in MS NAWM compared to control white matter tissues[1,9,10,12]. Extending the same thinking to the grey matter, gene changes have been studied in normal appearing motor cortex tissue isolated from MS brains and healthy controls[13]. In line with the demonstration of similar levels of myelin immunostaining, mRNAs of myelin genes were not significantly different between MS patients and controls. The results do, however, identify significant decrease in genes belonging to two broad biological categories: oxidative phosphorylation and GABAergic neurotransmission.

Firstly, in the category of oxidative phosphorylation, a significant reduction in mRNA of nuclear encoded mitochondrial genes was observed, translating into decreased protein expression. Decrease in both mRNA and protein expression was accompanied by functional reduction in respiratory chain complexes I and III. These results offer a mechanism for the axonal degeneration observed in progressive MS patients. Due to the redistribution of Na^+ channels and the resulting increased influx of sodium, ATP consumption is greatly increased in demyelinated axons[14,15]. To compensate for the higher energy demand, mitochondria migrate to the demyelinated axoplasm but have decreased respiratory capacity as found from the mRNA, protein and functional data. The combination of an axon with increased energy demands and a mitochondrial population less able to meet those demands may therefore trigger premature axonal degeneration. This imbalance in energy needs may be further compounded by the fact that, in addition to defects in the expression of nuclear encoded protein and mRNA, neurons in progressive MS, irrespective of the location and pathology, harbor increased levels of mitochondrial DNA deletions[18]. These results suggest that neuronal and axonal mitochondria pathology is prominent in MS tissues and may play a critical role in neuronal degeneration and MS pathogenesis.

In contrast to the predicted deleterious effect of the gene changes in the oxidative phosphorylation pathway, the changes in the GABAergic inhibitory neurotransmission

pathway may be beneficial. Reduced inhibitory innervations up-regulate neuroprotective pathways in murine cortex[19]. Decreased expression of the GABAergic genes coupled with increased expression of neurotrophic genes could therefore be part of the endogenous defense mechanism mounted by the MS brain. In the same dataset, increased ciliary neurotrophic factor (CNTF) signaling was found in motor neurons from non-lesioned motor cortex. CNTF is an established neurotrophic factor that enhances neuronal survival during development and in disease. Translational and transcriptional products of CNTF-related genes were quantified and localized in control and MS cortices[20]. An active and functionally significant role for CNTF in MS patients is supported by the report that MS patients with CNTF-null mutations have an earlier disease onset and a more aggressive disease course[21]. The microarray based expression profiling studies and downstream validations described here therefore provide important insights into the disease pathogenesis.

Gene expression studies in cortical lesions

Gene expression studies in cortical lesions have focused on mechanisms associated with either the formation of cortical lesions or remyelination. Classically, cortical lesions derived from autopsy studies have shown an absence of infiltrating inflammatory cells and no disruption of the blood-brain barrier. Recently, however, using biopsy materials from acute MS patients, inflammatory cortical lesions have been reported[22]. The pathogenic role of acute cortical lesions and the reasons they cannot be detected with routine MRI procedures are still to be ascertained.

Meningeal inflammation and B cell-like follicles, increasingly appreciated as pathological features of MS, have sometimes been associated with cortical lesions[23–25]. The role of meningeal B-cells in cortical pathology was explored in one of the first gene expression studies using cortical lesions[26]. Comparing cortical and meningeal tissues isolated from 6 MS and 8 control patients, significant increases in levels of IgG genes as well as IgG deposition were detected in the meningeal layers of MS brains. No genes were found to be different between lesioned and non-lesioned cortical samples, possibly due to the heterogeneity of the samples and/or batch effects interfering with the results. Despite the upregulation of IgG genes in the brains of MS patients, there was no corresponding upregulation of FcR genes, which bind to IgGs in white matter[26]. The authors concluded that the upregulation of IgG related genes and not FcRs probably reflected increased secretion of IgG from plasma cells, rather than B-cell accumulation[26]. Although some reports have detected the presence of Epstein Barr virus (EBV) in meningeal B-cells that correlate with cortical lesions[27,28], using PCR techniques on DNA isolated from brains of MS patients, no EBV sequences were detected. While there is a significant association between EBV and MS, the link between B cell-like follicles infected by EBV and cortical lesions remains to be established[29,30].

In a more recent study, the gene expression of one active and two chronic active MS cortical lesions was compared to cortical samples from three patients with tuberculous meningitis (representing an inflammatory meningeal condition), three with Alzheimer's disease (representing a neurodegenerative condition), and three controls[31]. Despite the use of formalin-fixed tissue for RNA measurements (offering less optimal sample quality compared to fresh-frozen tissues), the identified cortical gene changes were highly specific to MS. The comparison between the single acute cortical lesion and the remaining samples showed, among others, increased expression of immune related genes, which supports the rationale for further investigation of these extremely rare autopsy cases with active cortical lesions[22,31]. Comparing MS cortical tissues with all other samples (tuberculous meningitis, Alzheimer's disease and control cortex), several key biological pathways that could play a role in cortical MS pathology were identified including cell death, DNA damage, regulation

of transcription, cell survival, and axonal and synaptic biology. The biological category of oxidative damage was also identified and oxidized lipids were localized to apoptotic neurons[31]. The presence of apoptotic neurons has been previously described in cortical lesions[7] although the mechanisms that lead to neuronal death in MS still remain to be established.

The restoration of myelin, or remyelination, is among the best known neuroprotective strategies[1,32]. It is a spontaneous process that occurs both in experimental models and MS brains[33–38]. A recent study in MS brains found enhanced reparative capacity of cortical lesions compared to white matter lesions[39]. Direct comparison of remyelination in Type I (leukocortical) lesions found 86% greater remyelination in grey matter compared to white matter. Interestingly, this repair process appeared to be ongoing, as the number of actively-myelinating oligodendrocytes (oligodendrocytes within cortical lesions with PLP-positive cell bodies and processes extending to short internodes) in the cortex was six times greater than in white matter[39]. Increases in hyaluronan and associated molecules have been shown to inhibit maturation of oligodendrocyte progenitor cells and remyelination[38]. To analyze the difference in this propensity for remyelination, mRNA and protein levels of GFAP (glial fibrillary acidic protein), CD44, and versican (a hyaluronan-binding protein) were measured. Increases in mRNA and protein levels were detected in the white matter portion of leukocortical lesions compared to cortical portions. These findings suggest that cortical lesions have a more conducive environment for repair and remyelination[38,39]. Simultaneous analysis of cortical and white matter tissue could therefore reveal important regulators of remyelination in MS. In one such comparison, PCR-based screening of 92 phoshotyrosine phosphatase mRNAs revealed DUSPY15/VHY as an inhibitor of oligodendrocyte maturation[40], which is increased in MS lesions compared to controls. These studies support the utility of unbiased expression profiling as a reliable tool to understand molecular changes underlying cortical pathology.

Gene expression studies in MS hippocampus

The hippocampus is among the non-neocortical regions that are extensively demyelinated in progressive MS[41–43]. Cognitive dysfunction occurs in greater than 50% of MS patients, affecting episodic learning and memory, information processing speed, and working memory. Imaging and pathological studies support the possibility that hippocampal demyelination contributes to cognitive decline in MS patients[44,45]. Hippocampal demyelination is a prominent feature of post mortem MS brains, with 50–80% of hippocampi showing some degree of demyelination[41,42]. In our analysis, 12 of the 22 hippocampi analyzed were significantly demyelinated[43]. An encouraging observation was the retention of 80% or more of neurons in demyelinated hippocampi. This identifies the demyelinated hippocampal neuron as a promising therapeutic target. Despite the minimum neuronal loss, demyelinated hippocampi had a 40% decrease in synaptic densities and decreased expression of neuronal mRNA and proteins associated with axonal transport, glutamate neurotransmission, glutamate homeostasis, and memory and learning[43]. These data support the concept that demyelination alters neuronal gene expression and synaptic connectivity[43]. If reduced synaptic densities are a general consequence of demyelination, it could contribute to cortical atrophy and to the continuous neurological decline seen in the progressive stages of MS. Memory function was also impaired in an animal model of hippocampal demyelination[46] with the majority of gene changes similar to those measured in MS demyelinated hippocampus. In addition, gene regulatory microRNAs have been identified in MS and rodent hippocampus that were shown to target altered genes and change their mRNA expression following demyelination[46]. The results support the hypothesis that microRNAs may alter neuronal gene function following demyelination.. Using rodent model of hippocampal demyelination, it was shown that remyelination

abolished the memory dysfunction caused by demyelination[46]. Mechanistic insight into regulatory function of these miRNAs could lead to development of therapeutic options targeted to enhancing neuronal function.

Conclusions

The genetic complexity of MS provides an ideal platform for the application of gene expression profiling studies. The few gene-related studies using cortical tissues from MS brains provide evidence of mitochondrial and oxidative damage, impaired axonal transport, synaptic dysfunction, impaired neurotransmission, and increased cell death pathways in MS cortical neurons. Additionally, gene-related studies have also identified and validated increased neuroprotective pathways and remyelination efficiency in cortical lesions. This technology should now be applied specifically to compare pathological changes between normal and demyelinated cortex as well as between the relapsing-remitting and progressive disease courses. This is of paramount importance since the underlying mechanisms of cortical demyelination and associated changes are currently unknown. Cortical changes may also be retrogradely influenced by alterations in the spinal cord. Comparative gene changes in spinal cord coupled with proteomic approaches would therefore provide critical clues to disease progression and how downstream changes affect cortical pathology. Advancements in newer RNA sequencing studies to determine gene changes have also been applied to MS research[47]. These tools should now be applied to determine RNA changes in MS cortical neurons. Recognizing the limitations of any technology is important for successful implementation. Microarray data therefore need to be validated using independent methods, preferably in independent cohorts of samples, to reconfirm the results. Investigators should avoid experiments lacking downstream validation, or using, unsuitable or inadequately characterized biological material. Once quality control measures are followed, studies can be reliably compared between laboratories. Human tissue is not amenable to manipulation, thus efforts should be directed towards the development of animal models that mimic the cortical pathology observed in MS patients. Future research into regulatory factors such as transcription factors, microRNAs and epigenetic modifications that control gene expression in MS should also be undertaken. Finally, integration of pathology, neuroimaging and genomic research will be critical in having a comprehensive understanding of the cortical pathology in MS.

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Table 1

Summary of gene profiling studies in grey matter tissue

TISSUE	BRAIN AREA/SAMPLE TYPE	COMPARATIVE TOOL	Q-PCR	FINDING	
6 MS (SP, PP) 6 Ctl	Motor cortex Frozen	Affymetrix HG-U133A,B ~39,000 probes	Yes	Non-lesioned motor cortex from MS brains was compared to control motor cortex. Major findings include decreases in genes related to mitochondrial function and inhibitory neurotransmission. These results provide a mechanism of axonal degeneration through energy imbalance. In the same dataset, genes from the CNTF-mediated neuroprotective signaling pathway were increased, possibly reflecting an endogenous neuroprotective response.	**13** **20**
6 MS (SP, PR, NA) 8 Ctl	Cerebral Cortex Frozen	Applied Biosystems Human Genome Survey Microarray v1.0 ~31,700 probes	Yes	Non-lesioned and lesioned cortex from MS brains was compared to control cortex. No genes were found to be significantly different between MS lesioned and non-lesioned cortex. Compared to controls, 532 genes were altered in MS cortex. Major findings of the study include increased mRNA levels of IgG-related genes, possibly due to meningeal plasma cells that are not infected by Epstein-Barr virus.	**26**
8 MS (SP, PP) 4 Ctl	Hippocampus Frozen	Affymetrix HG-U133A ~13,000 probes	Yes	Non-lesioned MS hippocampus was compared to lesioned MS and control hippocampus. There were 799 genes significantly altered between non-lesioned and lesioned hippocampus. A significant loss of neurons was not reported. Major findings of the study include decreases in mRNA and proteins belonging to axonal transport, glutamate neurotransmission, synaptic maintenance and memory/learning.	**43**
3 MS (SP) 3 Ctl	Frontal Cortex Frozen	Custom PCR array containing 92 Protein Tyrosine Phosphatases (PTPs)	NA	PTP levels in white matter and grey matter non-lesioned and lesioned tissues were compared. 52 PTPs were altered in MS white matter compared to 9 in grey matter. The study identified VHY/Dusp15 as a regulator of oligodendrocyte differentiation.	**40**
3 MS (SP) 3 TM 3 AD 3 Ctl	Frontal/Parietal/Temporal cortex Paraffin embedded	Agilent-Whole-Human Genome Microarrays ~ 41000 probes	Yes	Cortical lesions were divided into 1 active lesion and 2 chronic active lesions. MS cases were compared to all remaining samples (Tuberous meningitis, Alzheimer's disease and control). One of the major aspects of this study was identifying 109 genes specific to MS and belonging to inflammation, cell death, DNA repair, transcription and translation. Although statistical analysis could not be performed, comparison of one single MS case (active lesion) with remaining samples (MS cortex, Tuberculous meningitis, Alzheimer's disease and control cortex) led to changes in similar biological categories.	**31**

SP: secondary progressive MS; PP: Primary progressive MS; Ctl: control; PR: Progressive relapsing; NA: not available TM: Tuberculous meningitis AD: Alzheimer's disease; Q-PCR: Quantitative polymerase chain reaction