

GENE EXPRESSION IN TEMPORAL LOBE EPILEPSY

Gene Expression in Temporal Lobe Epilepsy Is Consistent with Increased Release of Glutamate by Astrocytes. Lee TS, Mane S, Eid T, Zhao H, Lin A, Guan Z, Kim JH, Schweitzer J, King-Stevens D, Weber P, Spencer SS, Spencer DD, de Lanerolle NC. *Mol Med.* 2007;13(1–2):1–13. Patients with temporal lobe epilepsy (TLE) often have a shrunken hippocampus that is known to be the location in which seizures originate. The role of the sclerotic hippocampus in the causation and maintenance of seizures in temporal lobe epilepsy (TLE) has remained incompletely understood despite extensive neuropathological investigations of this substrate. To gain new insights and develop new testable hypotheses on the role of sclerosis in the pathophysiology of TLE, the differential gene expression profile was studied. To this end, DNA microarray analysis was used to compare gene expression profiles in sclerotic and non-sclerotic hippocampi surgically removed from TLE patients. Sclerotic hippocampi had transcriptional signatures that were different from non-sclerotic hippocampi. The differentially expressed gene set in sclerotic hippocampi revealed changes in several molecular signaling pathways, which included the increased expression of genes associated with astrocyte structure (glial fibrillary acidic protein, ezrin-moesin-radixin, palladin), calcium regulation (S100 calcium binding protein beta, chemokine (C-X-C motif) receptor 4) and blood-brain barrier function (Aquaaporin 4, Chemokine (C-C- motif) ligand 2, Chemokine (C-C- motif) ligand 3, Plectin 1, intermediate filament binding protein 55kDa) and inflammatory responses. Immunohistochemical localization studies show that there is altered distribution of the gene-associated proteins in astrocytes from sclerotic foci compared with non-sclerotic foci. It is hypothesized that the astrocytes in sclerotic tissue have activated molecular pathways that could lead to enhanced release of glutamate by these cells. Such glutamate release may excite surrounding neurons and elicit seizure activity.

COMMENTARY

Hippocampal sclerosis is a very common feature of temporal lobe epilepsy. It is found in approximately 50 to 75 percent of temporal lobe resections performed to relieve medically intractable limbic epilepsy; the presence of sclerosis is a good indicator for a positive outcome to surgery. Since most of the epilepsy surgery resections consist of temporal lobectomies, electrophysiological and neuropathological investigations of epileptogenesis usually are performed on hippocampus and surrounding temporal cortex. Sclerosis is characterized by gross anatomical and microscopic changes, but the main histological feature is widespread neuronal cell death and gliosis/astrocytosis. As with many pathological findings in neurodegenerative diseases, it is difficult to determine if the changes are a cause or consequence of epileptic seizures. Studies with animal models suggest that hippocampal sclerosis is not necessary for progression to epileptic seizures (1); however, the clinical evidence summarized in the article by Lee et al. suggests a crucial role for the sclerotic hippocampus in determining seizures. If this is the case, then comparing the molecular properties of sclerotic with nonsclerotic hippocampi may provide insight into the cause of seizures in temporal lobe epilepsy.

Modern molecular tools allow genome-wide gene expression analyses to be performed on minute amounts of tissue. One of the first applications of the “gene chip” in human epilepsy was to investigate the correlates of multiple antiepileptic drug resistance (2). Subsequent studies explored epileptogenesis in animal models (3). Gene chip analysis of tissue isolated from animal models of epilepsy has several advantages, including relative low cost of otherwise impractical genome-wide comparisons, availability of impressive data analysis tools to analyze patterns of gene expression, and completion of the human genome project, allowing for cross comparison with patients’ data. In contrast, limitations of animal models include the difficulty of validating thousands of data points as well as reproducibility and correlation with data from different laboratories. As in all studies of epileptic human brain, true control brain tissue is not readily available. Recognizing these limitations, Lee and colleagues set out to use gene chips to analyze gene expression changes in hippocampal tissue obtained from surgical resections. Pitfalls associated with control tissue and interindividual variability were largely prevented by comparing hippocampi removed solely from patients with epilepsy and by focusing on the differences seen between sclerotic and nonsclerotic hippocampi of the same patients. This approach produced a number of results that were then validated with routine immunocytochemical and reverse transcriptase polymerase chain reaction approaches.

The title of this article does not tell the whole story of what the authors found. In fact, gene expression differences that were

unveiled have more to do with inflammation, vascular changes, and cell cycle/apoptosis than with glutamate uptake or transport by astroglia. These changes cannot be assumed to be exclusively astrocytic, since whole tissue mRNA was used. Furthermore, immunocytochemical detection revealed changes in other cell types, such as vascular endothelial cells. How whole tissue data differ from mRNA isolated from specific cell components has been discussed elsewhere (4). The exact analysis of cytological correlates of the mRNA data also leads to a potential pitfall with the interpretation of data. For tissue comparison, the authors defined gene expression levels of greater or smaller than 50% as a significant change; a definition that possibly leads to an overinterpretation of results, because cell numbers and transcriptional activity were not controlled for. Controlling for transcriptional activity is particularly important for gene expression levels that are not adjusted by any cellular quantification, such as number of astrocytes, ratio of astrocytes/oligodendrocytes/neurons, and contribution of nonbrain or vascular cells. The contributions of vascular cells are particularly important because several of the inflammatory markers and antigens that were reported to be upregulated are expressed by white blood cells, which may extravasate after seizures, leading to hippocampal sclerosis.

One of the unexpected and most experimentally solid findings was that the use of an unsupervised hierarchical cluster analysis was able to organize all samples into two major groups: one constituted specimens isolated from sclerotic hippocampi and the other containing mRNA from temporal lobe epilepsy hippocampi, not characterized by sclerosis. The surprising fact was not so much that sclerotic tissue had a distinct gene expression profile, but rather that sclerotic hippocampi as a group had similar gene expression profiles, regardless of whether the sclerosis was mass-associated (i.e., peritumoral) and nontumoral. The infiltrative nature of some of the tumors (e.g., glioma, oligodendroglioma) and the ischemic or cystic origin of other samples may have predicted a significant difference in mRNA, but this was not the case.

Sclerotic hippocampi demonstrated increased expression of several astrocytic genes, but it was not immediately clear if the finding was a reflection of an increased number of cells or increased transcriptional activity. A few of the upregulated genes that the investigators attributed to astrocytes, in fact, are expressed in many cell types and diseases, other than epilepsy. For instance, the protein S100A6 is upregulated in Alzheimer's and amyotrophic lateral sclerosis, CD44 antigen is expressed in bone marrow-derived cells, and the gene product AHNAK is expressed in blood-brain barrier endothelium. Indeed, a large number of upregulated genes were associated with blood-brain barrier function or systemic inflammation, further supporting a significant role for these two mechanisms in the etiology of seizures (5–7). One of the upregulated genes, *CCL3*, whose macrophage inflammatory protein is involved in the acute in-

flammatory state occurring with the recruitment and activation of polymorphonuclear leukocytes, suggests the presence of an infectious agent in sclerotic tissue. Similarly, interleukin-11 is a mediator of bone marrow-derived cells involved in the immune response. Additional findings are related to the expected loss of mRNA signaling, resulting from widespread neuronal cell death characteristic of sclerotic brain.

The introductory statements by Lee et al. support a role for sclerosis in the pathogenesis of hippocampal epilepsy; yet, the data presented do not clarify which of the observed changes are pathogenic and which are simply the result of seizures. Similarly, it is important to understand whether sclerosis is caused by some of these gene expression changes or is the trigger for altered inflammatory, glial, and cerebrovascular expression profiles. One of the unavoidable pitfalls of static, neuropathological studies is the lack of temporal perspective. For example, inflammation may have preceded sclerosis, and gliosis may be a consequence of neuronal cell loss. Conversely, neuronal death may be a consequence of seizures, which may also constitute the initiating factor for a mopping up intervention by immune cells, including macrophages. Unfortunately, the results presented here do not provide a major leap in understanding the origin of temporal lobe epilepsy, hippocampal seizures, or whether hippocampal sclerosis is a consequence or cause of seizures. This study, however, provides additional support for an immunological component in the pathophysiology of temporal lobe epilepsy and hippocampal sclerosis.

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References

1. Jefferys JG. Hippocampal sclerosis and temporal lobe epilepsy: cause or consequence? *Brain* 1999;122(Pt 6):1007–1008.
2. Dombrowski S, Desai S, Marroni M, Cucullo L, Bingaman W, Mayberg MR, Benghez L, Janigro D. Overexpression of multiple drug resistance genes in endothelial cells from patients with refractory epilepsy. *Epilepsia* 2001;42:1504–1507.
3. Ess KC, Uhlmann EJ, Li W, Li H, Declue JE, Crino PB, Gutmann DH. Expression profiling in tuberous sclerosis complex (TSC) knockout mouse astrocytes to characterize human TSC brain pathology. *Glia* 2004;46:28–40.
4. Marroni M, Kight KM, Hossain M, Cucullo L, Desai SY, Janigro D. Dynamic in vitro model of the blood-brain barrier. Gene profiling using cDNA microarray analysis. *Methods Mol Med* 2003;89:419–434.
5. Vezzani A, Granata T. Brain inflammation in epilepsy: experimental and clinical evidence. *Epilepsia* 2005;46:1724–1743.
6. Marchi N, Angelov L, Masaryk T, Fazio V, Granata T, Hernandez N, Hallene K, Diglaw T, Franic L, Najm I, Janigro D. Seizure-promoting effect of blood-brain barrier disruption. *Epilepsia* 2007;48(4):732–742.
7. Oby E, Janigro D. The blood-brain barrier and epilepsy. *Epilepsia* 2006;47:1761–1774.