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Hippocampal Proteomic Profile in Temporal Lobe Epilepsy*

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ABSTRACT

In this study we used proteomics approaches to obtain the protein profile of human epileptic hippocampi (N=6) and control hippocampi obtained from autopsy (N=6). We used two-dimensional gel electrophoresis (2-D) coupled to HPLC and Mass spectroscopy (MALDI-TOF) to identify proteins differentially expressed. Nine proteins were differentially expressed comparing the hippocampus of patients with the hippocampus of control. Proteins that were increased in the hippocampus of patients with TLE were: isoform 1 of serum albumin, HSP 70, dihydropyrimidinase-related protein 2, isoform 1 of myelin basic protein, proton ATPase catalytic subunit A, and dihydrolipoyllysine-residue acethyltransferase component of pyruvate dehydrogenase complex. The expression of isoform 3 of spectrin alpha chain (fodrin) was down-regulated while the proteins glutathione S-transferase P and the DJ-1 (PARK7) were detected only in the hippocampus of patients with TLE. Taken together, our results provide evidence supporting a direct link between metabolic disturb and oxidative damage related to pathophysiology of TLE. Besides, indicates biomarkers for further investigations as therapies targeted to epilepsy.

Keywords: temporal lobe epilepsy, hippocampus, proteomics, biomarkers.

RESUMO

Perfil proteômico hipocampal em epilepsia do lobo temporal

No presente estudo empregou-se o método de proteômica para obter a expressão diferencial de proteínas em hipocampo de pacientes com epilepsia do lobo temporal (ELT) (N=6) comparado a hipocampos controle obtidos por meio de autópsia (N=6). O estudo foi feito por meio de eletroforese bidimensional, acoplada a HPLC e espectroscopia de massa. As proteínas que tiveram expressão aumentada no hipocampo de pacientes com ELT foram: isoforma-1 da soroalbumina, HSP70, diidropirimidinase-2, isoforma-1 da proteína básica da mielina, subunidade catalítica A da próton ATPase, glutationa S-transferase P, proteína DJ-1 (PARK7), e resíduo diidropolilisina do componente acetil-transferase do complexo da piruvato desidrogenase. A expressão da isoforma-3 da cadeia alfa da espectrina (fodrina) foi menor no hipocampo de pacientes com epilepsia do lobo temporal e as proteínas glutationa S-transferase P e PARK7 foram detectadas somente no tecido epiléptico. Assim, nossos resultados trazem evidencias sobre a direta relação entre distúrbio metabólico e dano oxidativo na patofisiologia da ELT. Além disto, o estudo indica biomarcadores para futuras investigações como alvos terapêuticos para epilepsia.

Palavras-chave: epilepsia do lobo temporal, hipocampo, proteômica, biomarcadores.

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BACKGROUND AND PURPOSE

Epilepsy is a chronic disorder characterized by repeated seizures resulting from abnormal activation of neurons in the brain. There are some studies analyzing gene expression in epilepsy. However there is a lack of studies evaluating the regulation of the gene expression, in post-transcriptional or post-translational level, and these data are very important to elucidate the identify proteins which are involved in the pathophysiology of epilepsies. We have used proteomic approaches to investigate the expression of soluble proteins in the hippocampi of patients with temporal lobe epilepsy.

MATERIAL AND METHODS

Human tissue

All experiments were performed under approval from the Institutional Ethics Committee of the Universidade Federal de São Paulo (UNIFESP). Patients with intractable epilepsy were submitted to standard cortico-amygdalo-hippocampectomy at the Hospital of São Paulo (UNIPETE/SPDM-UNIFESP). Selected patients (N=6) had detailed anamnesis, video-EEG recordings and MRI studies and after these procedures the epileptogenic zone was delineated. Control hippocampi were obtained from brains showing no evidence of pathology on the basis of gross and routine histological examination removed from autopsies made by a pathologist from Dept. Pathology, Incor/FMUSP.

Sample preparation

Hippocampi were mixed with homogenization buffer consisting of 7M urea, 2M thiourea, 4% (w/v) CHAPS, 10 mM DTT, 1mM EDTA, 1mM PMSF, 0.2mM Na_2VO_3 and 1mM NaF. After sonication on an ice bath, the suspension was centrifuged at 12000 × g for 40 min at 4°C. The protein content of the supernatant was determined by using the Bradford method.

Two-dimensional gel electrophoresis (2-DE)

Five hundred micrograms of protein from each sample were used for 2-DE. 17 cm linear pH 3-10 IPG strips (BioRad Laboratories, Hercules CA, USA) were used for the first dimension. Isoeletric focusing was performed by using a Protean IEF cell (BioRad Laboratories, Hercules, CA, USA). Focusing was started at 100 V, and after the voltage was gradually increased to 8 kV in a linear mode during 150 min and, finally, 8.000 V was applied until 45 kVh was reached. The equilibrated strips were then placed onto second dimension 12% SDS-PAGE gels. The SDS-PAGE was conducted in a standard Tris-Glycine-SDS buffer in Protean II xi Cell (BioRad Laboratories, Hercules, CA, USA) at a constant current setting of 20 mA/gel for 1h, then at 60 mA/gel until the end. Gels were stained by Coomassie method (Candiano et al., 2004).

Image analysis and determination of proteome differences

Stained gels were scanned by a GS-800 calibrated densitometer (BioRad Laboratories, Hercules, CA, USA) and PDQuest 2D-gel analysis software (Version 8.0.1, BioRad Laboratories, Hercules, CA, USA) was used to match and analyze visualized protein spots among differential gels in order to compare protein content between TLE samples and control (N=6/group). Normalized density values were used for comparisons, and spots exhibiting at least 2 fold increases or decreases were identified by mass spectrometry.

In-gel digestion

Excised protein spots were subjected to in-gel trypsin digestion for 20 min at room temperature. The reaction was stopped by adding 50 μl of a solution of trifluoroacetic acid (TFA) at 0.1%. The samples were dehydrated in a vacuum centrifuge and resuspended in 0.1% TFA until ready to be applied to LC-ESI-MS/MS analysis.

Nano-LC-ESI-MS/MS analysis

An aliquot (4.5 μl) of digested proteins was inject by analytic column C18, 1.7 μm, BEH 130 RP-UPLC (nanoAcquity UPLC, Waters) coupled with nano-electrospray tandem mass spectrometry on a Q-Tof Ultima API mass spectrometer (MicroMass/Waters). The gradient was 0-50% acetonitrile in 0.1% formic acid over 45 min. Databases searches for peptide identification from LC MS-MS experiments were done with Mascot Distiller v.2.3.2.0, 2009 (Matrix Science, Boston, MA) using carbamidomethyl-cys as fixed modification, lysine and/or arginine methylation, lysine acetylation, methionine and/or tryptophan oxidation as variable modification and 0.1Da MS and MS/MS fragment tolerances. Proteins were identified by searching a *Homo sapiens* protein database.

STATISTICS

All data were presented as means ±SD and analyzed by Student's *t*-test. Statistical significance was defined as $p<0.05$.

RESULTS

In total, twelve 2-DE gels corresponding to 6 patients and 6 controls were analyzed simultaneously and matched in the same set. We detected approximately 192-269 spots in each gel. The image analysis revealed 215±21 spots in the patients, and 237±32 in the control. When 2-DE patterns

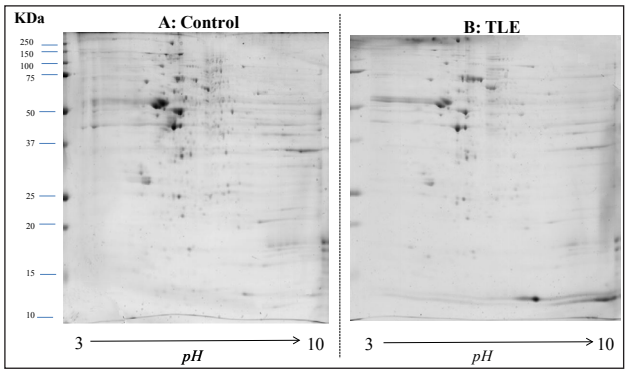

Figure 1. Two-dimensional gel electrophoresis patterns of hippocampus of control (**A**) and TLE (**B**) groups (N=6/group).

of the groups were compared 16 spots were differentially expressed between control and epileptic groups.

The Figure 2 describes unique and overlapping proteins with significant difference between the groups. In the epileptic group 6 spots were up-regulated and 3 were present only in this group. In the control group 2 spots were up-regulated and 5 were found only in this group.

As shown in Table 1, a total of nine distinct proteins were finally identified with ESI-Q-TOF MS and MS/MS analysis. Identified proteins were: isoform 1 of serum albumin, HSP 70, dihydropyrimidinase-related protein 2, isoform 1 of myelin basic protein, isoform 3 of Spectrin alpha chain, proton ATPase catalytic subunit A, glutathione S-transferase P, protein DJ-1 (PARK7), and dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex. The expression of Spectrin was down-regulated in the hippocampi of patients with temporal lobe epilepsy. Glutathione S-transferase P and PARK7 were detected only in epilepsy. All other proteins were up-regulated in epilepsy. Table 2 shows the principal cellular and molecular functions related to the identified proteins.

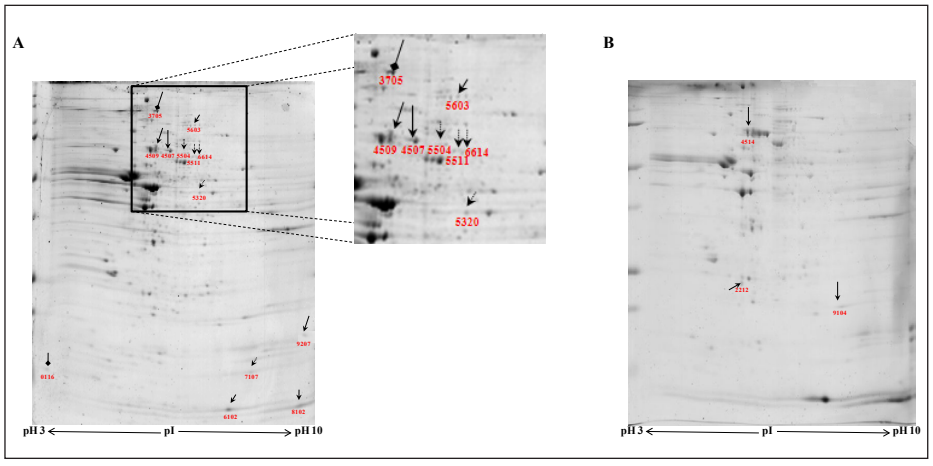

Figure 2. Two-dimensional gel electrophoresis analysis for the validation of protein expression differences between control and TLE groups. In **A**: up-regulated spots in the TLE are shown as filled arrows (6), marked with dotted arrows are the spots detected only in the control group (5), and marked with arrows with square tip are the spots up-regulated in the control group (2). In **B**: marked with arrows are the spots detected only in the epileptic group (3). Significance: ≥2 fold change, *t*-test, $p<0.05$.

Table 1. Protein differentially expressed in the hippocampus of patients with TLE.

IP	Protein name	MW	Changes
5.92	Isoform 1 of Serum albumin – ALB	71317	↑
5.56	Heat shock-related 70 kDa protein 2 – HSPA2	70263	↑
8.2	Dihydropyrimidinase-related protein 2 – DPYSL2	77912	↑
9.79	Isoform 1 of Myelin basic protein – MBP	33097	↑
5.21	Isoform 3 of Spectrin alpha chain, brain – SPTAN1	282906	⇩
5.35	V-type proton ATPase catalytic subunit A – ATP6V1A	68660	↑
5.43	Glutathione S-transferase P – GSTP1	23569	+
6.33	Protein DJ-1 – PARK7	20050	+
7.96	Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial – DLAT	69466	↑

MW: molecular weight; IP: isoelectric point.
All identifications met statistical confidence criteria according to MASCOT and NCBI scoring schemes ($p<0.05$).

Table 2. Cellular and molecular functions of identified proteins.

Protein name	Function
Isoform 1 of Serum albumin – ALB	Regulation of the colloidal osmotic pressure of blood. Major zinc transporter in plasma.
Heat shock-related 70 kDa protein 2 – HSPA2	Chaperone.
Dihydropyrimidinase-related protein 2 – DPYSL2	Necessary for the remodeling of the cytoskeleton after injury. Plays a role in axon guidance, neuronal growth cone collapse and cell migration.
Isoform 1 of Myelin basic protein – MBP	Strutural constituent of myelin sheath. It has a role in both its formation and stabilization. The smaller isoforms might have an important role in remyelination of denuded axons in multiple sclerosis.
Isoform 3 of Spectrin alpha chain, brain – SPTAN1	Interacts with calmodulin in a calcium-dependent manner. Candidate for the calcium-dependent movement of the cytoskeleton at the membrane.
V-type proton ATPase catalytic subunit A – ATP6V1A	Responsible for acidifying a variety of intracellular compartments in eukaryotic cells. It may participate in maintenance of cytoplasmic Ca^{2+} homeostasis.
Glutathione S-transferase P – GSTP1	Conjugation of reduced glutathione to exogenous and endogenous hydrophobic electrophiles.
Protein DJ-1 – PARK7	Protects cells against oxidative stress and cell death.
Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial – DLAT	Catalyzes the overall conversion of pyruvate to acetyl-CoA and CO_2. It contains multiple copies of three enzymatic components: pyruvate dehydrogenase (E1), dihydrolipoamide acetyltransferase (E2) and lipoamide dehydrogenase (E3).

The function of each protein was identified using the UniProt database.

CONCLUSIONS

Taken together, our results provide evidence supporting a direct link between metabolic disturb and oxidative damage to the pathology of TLE. Besides, indicates biomarkers for further investigations as therapies targeted at epilepsy. The DPYSL2 a protein that is up-regulated in the hippocampus of epileptic patients with pharmacoresistant seizures has been associated with psychiatric disorder such as schizophrenia. Further studies are needed to elucidate whether this protein represent a link between TLE and psychiatric disorder or is only an independent mechanism. Reduction in fodrin expression is other important data from this study. Fodrin plays an important role in anchoring NMDA receptors in the membrane and disturbances in the expression of this protein may cause changes in the electrophysiological functioning of the receptor.

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REFERÊNCIA

Candiano G, Bruschi M, Musante L, Sabntucci L, Ghiggeri GM, Carnemolla B, Orecchia P, Zardi L, Righetti PG. Blue silver: a very sensitive colloidal Coomassie G-250 staining for proteome analysis. Electrophoresis. 2004;25:1327-33.

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