2DE-DIGE Proteomic Analysis in Mesial Temporal Lobe Epilepsy

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Epilepsy affects 1% to 2% of the general population; therefore, it is considered a public health problem by the World Health Organization. Mesial temporal lobe epilepsy (MTLE) is the most common and severe type of partial epilepsy, representing ~50% of all adult epilepsy patients and frequently associated with pharmaco-resistance. The relationship between MTLE and hippocampal sclerosis (HS) is well established. However, the precise pathogenesis of HS and its relationship with MTLE is not completely clarified. Two-dimensional electrophoresis (2DE) is a powerful fractionation method for complex protein mixtures. In difference gel electrophoresis (DIGE) based proteomics, the experimental and control samples are labeled with different fluorophores and are run in the same gel, thereby minimizing gel preparation variation. DIGE is one of the few techniques that is capable to perform quantitative proteomics, generating statistical data to differences in protein abundances. We analyzed the proteome of three hippocampus removed from patients with refratory MTLE who underwent epilepsy surgery. 2DE-DIGE identified 2 up-regulated and 10 down-regulated proteins as determined by Student's T-test ($p \leq 0.01$). The observed molecular weight of the spots ranged from 28 to 93 kDa. The identity of these spots will be elucidated by mass spectrometry in order to gain additional information in a global scale about the mechanism of epileptogenesis in MTLE.

Methods

1 – Experimental Design

Samples:
T1 Patient 1 ♂ 37 y.o.
C1 Control 1 ♂ 58y.o.

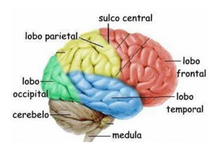
T2 Patient 2 ♂ 41 y.o.
C2 Control 2 ♂ 62 y.o.

T3 Patient 3 ♂ 38 y.o.
C3 Control 3 ♂ 55 y.o.

Gel distribution:

	Cy2	Cy3	Cy5
Gel 1	pool	C1	T1
Gel 2	pool	T2	C2
Gel 3	pool	C3	T3

2 – Protein extraction



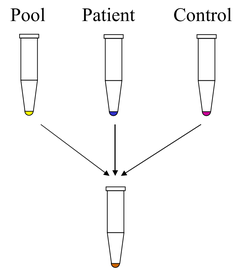
Human hippocampus

Total protein extraction
Using polytron

Soluble fraction

pH 8,0 ~ 9,0

3 – CyDye DIGE Fluor minimal labeling



400 pmol fluorescent dye
+ 50 µg total protein extract

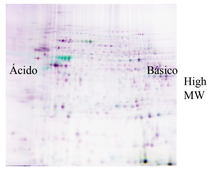
30' at 4ºC in dark room

Stop reaction with L-lisin

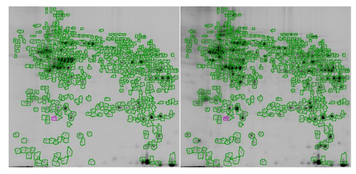
4 – Isoelectric focalization (1st dimension)

10h at 20ºC
1h at 500V
1h at 1000V
Total of 55000V

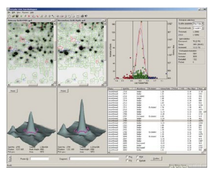
5 – SDS-PAGE (2 nd dimension)



6 – Imaging acquisition and statistical analysis (Typhoon)



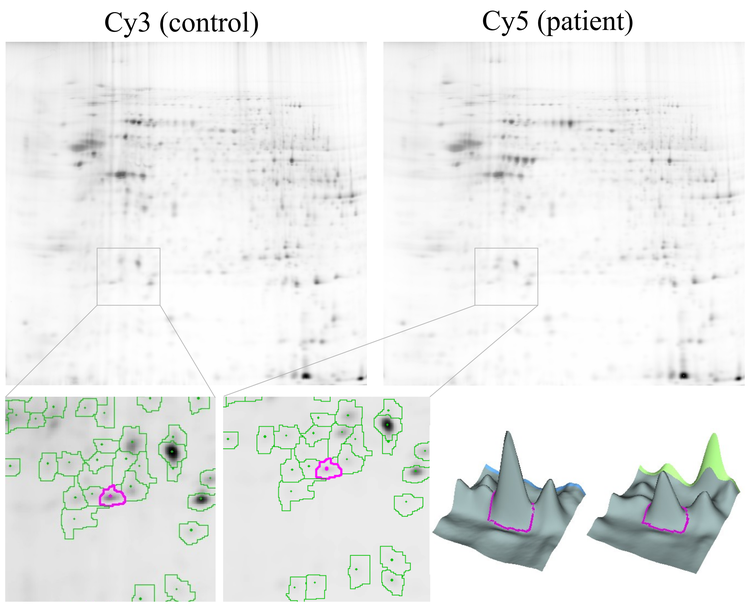
Spot detection



DeCyder 2D Differential Analysis Software v. 6.5

Results

Cy3 (control) Cy5 (patient)

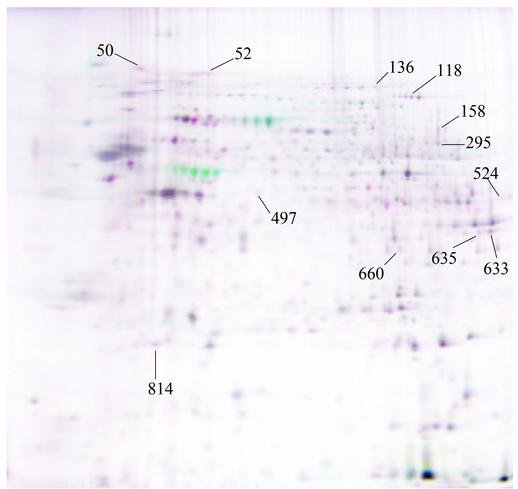


Spots nº 814

Protein Table T-test and Av.Ratio: Control / Treated

Pos.	Master No.	T-test	Av. Ratio	MW
1	50	0.00041	2.00	~93
2	660	0.00060	1.41	~35
3	158	0.0014	-1.24	~70
4	52	0.0025	1.51	~90
5	635	0.0036	1.38	~40
6	814	0.0056	1.44	~28
7	633	0.0060	1.37	~40
8	497	0.0061	1.48	~50
9	524	0.0066	1.41	~50
10	295	0.0076	1.31	~70
11	136	0.0083	-3.55	~70
12	118	0.0095	1.31	~90

Table with spots identified



Spot localization into 2D gel

Conclusions

- Protein extraction and staining was successful;
- We identified 12 spots differentially expressed in patients and controls;
- Mass spectrometry analysis of the 12 spots identified is under way.

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