EXPRESSION OF INFLAMMATION-ASSOCIATED MiCRORNAS IN EPILEPSY

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Background

Mesial Temporal Lobe Epilepsy with Hippocampal Sclerosis (MTLE-HS) is one of the most common pharmaco-resistant epileptic syndromes. Increasing evidence supports the involvement of inflammatory processes in the deregulation of neurotransmission that characterizes epilepsy (Gorter et al. 2006). Studies in animal models demonstrated that after acute seizures a rapid induction of cytokines and glial activation is involved in epileptic activity (Vezzani et al. 2011, Ravizza et al. 2006). It has been shown that these cytokines contribute to seizure-related hippocampal pathology, such as neuronal death, reactive gliosis and mossy fiber sprouting. These findings are corroborated by observations showing that cytokines (e.g.: IL-1β and TNFα) and cytokines receptors are overexpressed in patients' brain tissue (Vezzani et al. 2008). Gene expression may be regulated by several factors including small non-coding RNA molecules - MicroRNAs (miRNA) that control different biological process including immune system homeostasis and function. It has been showed that the activation of pro-inflammatory pathways via Toll Like Receptors, with the consequent expression of cytokines such as IL-1β, leads to the induction of miRNAs such as miR-155 and miR-146a. MiR-155 has been associated with the suppression of negative regulators of inflammation and upregulation of pro-inflammatory cytokines such as TNF-α whilst miR-146a acts in a feedback loop being a dominant negative regulator of inflammatory responses. Several evidences, both in patients and animal models, have demonstrated an abnormal brain expression of these miRNA in MTLE (Jimenez-Mateos et al. 2013, Omran et al. 2012, Aronica et al. 2010) . It is known that miRNA expression is very stable in biological fluids such as plasma or serum what makes them suitable biomarkers. Nevertheless the expression of these molecules in serum from MTLE patients is still poorly addressed.

Aim: To characterize miR-146a and miR-155 expression in serum of MTLE patients.

Patients and Methods

Fifty MTLE-HS patients and 46 healthy individuals were investigated. The study was approved by the Hospital Ethical Committee and all individuals gave written informed consent in accordance with Declaration of Helsinki. Total RNA was isolated from serum using the miRNeasy Mini kit (Qiagen) and reverse-transcribed with TaqMan miRNA Reverse Transcription Kit following the manufacturer's protocol (Applied Biosystems, Inc., Foster City, CA). TaqMan miRNA assay included specific RT Primers and TaqMan Probes to quantify mature microRNAs (has-miR-146a, has-miR-155). For normalization, RNU48 (hsa-miR-RNU48) was used. Each reaction was performed in triplicate and the average Ct value was used in analysis. Relative expression values were calculated using the $2^{-\Delta\Delta Ct}$ method. Differences in ΔCt were evaluated using two-tailed Student's t-test. Analyses were done with SPSS v.22 software and significant levels were set at $p<0.05$.

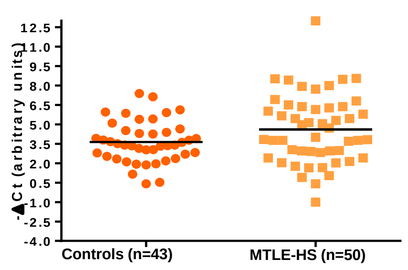
Table 1:Demographic and clinical characteristics of epileptic patients

Clinical and demographic characteristics	Patients
Sex (F/M)	27 / 23
Age, years ± SD	42 ± 12
Epilepsy age of onset, years ± SD	14 ± 11
Febrile Seizures Antecedents	24

Results

miR-155

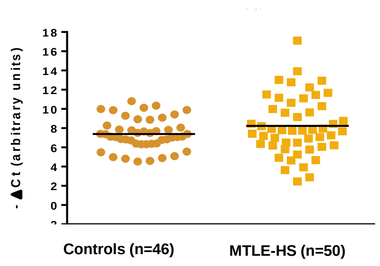
Figure 1: miR-155 Relative expression in MTLE-HS and controls



MTLE-HS patients had higher miR-155 expression than controls

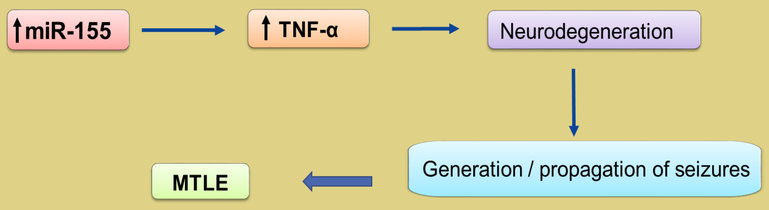
miR-146a

Figure 2: miR-146a Relative expression in MTLE-HS and controls



MiR-146a expression was 2 fold higher in MTLE-HS patients

Discussion



miR-155 expression is directly correlated with expression of TNF- α

Overexpression of TNF-α is associated with exacerbation of inflammatory response

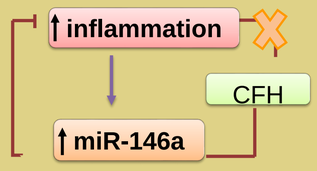
Exacerbation of inflammation may lead to the generation and/or propagation of seizures

Hennessy *et al.* 2015; Ashhab *et al.* 2013

miR-146a acts on a negative feedback loop: ↓IL-1β

↑ miR-146a is a compensatory mechanism to overcome the exacerbated inflammatory response

Aronica *et al.* 2010



CFH (Complement Factor H) is an inhibitor of inflammatory pathways

↑ miR-146a →↓ CFH → → exacerbation of inflammatory reaction

Lukiw *et al.* 2008; Boon *et al.* 2009

Final remarks

The induction of miRNA and their different effects may: - represent different cell responses during the several phases of epileptogenic process

- be the reflex of an abnormal function due to changes in the metabolic state of the cells

The role of miR-155 and miR-146a in epileleptogenic mechanism remains unknown and may depend on the availability of other molecules;

Two question are raised by these results:

Are miR-146a and /or miR-155 suitable biomarkers for MTLE-HS?

May these microRNAs be potential new therapeutic target?

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