Expression of miR-146a, an inflammation-associated microRNA, in Mesial Temporal Lobe Epilepsy

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Purpose

Mesial Temporal Lobe Epilepsy with Hippocampal Sclerosis (MTLE-HS) is one of the most pharmaco-resistant epileptic syndromes. Increasing evidence supports the involvement of immune and 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 and cytokines receptors are overexpressed in brain tissue of patients (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.

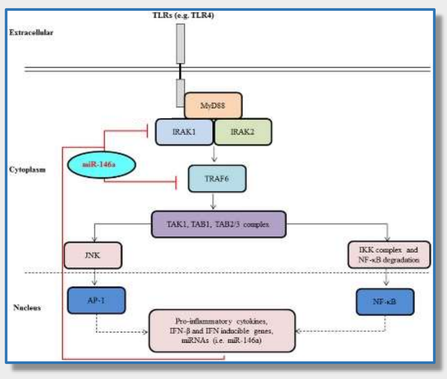


Fig 1 - MiR-146a negatively regulates signal transduction pathways leading inflammation(adapted from Saba *et al.* 2014)

It has been showed that the activation of pro-inflammatory pathways via TLRs with the consequent expression of cytokines such as IL-1β leads to the induction of miR-146a. This miR acts in a feedback loop being a dominant negative regulator of inflammatory responses (Figure 1). Several evidences, both in patients and animal studies, have demonstrated an abnormal brain expression of miR-146a in MTLE (Jimenez-Mateos *et al.* 2013, Omran *et al.* 2012, Aronica *et al.* 2010) .

Aim: Knowing that miRNA expression is very stable in biological fluids such as plasma or serum our aim was to characterize miR146a expression in serum of MTLE patients.

Methods

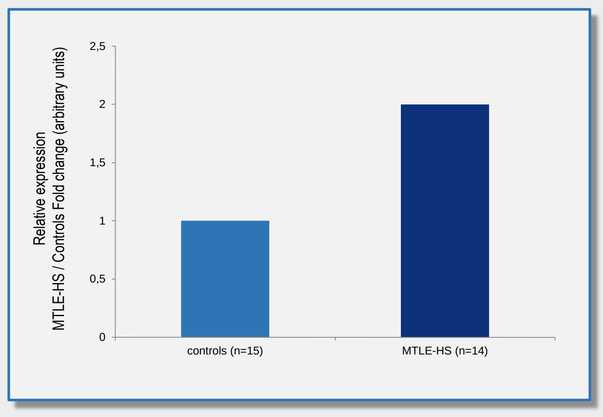
Fourteen MTLE-HS patients (6F/8M, mean age=44 ± 10.5 years) and 15 healthy individuals were studied. Total RNA was isolated from serum using the miRNeasy Mini kit (Qiagen) and reverse-transcribed with TaqMan miRNA Reverse Transcription Kit following the manufacturer's protocol (Applied Biosystems, Inc., Foster City, CA). TaqMan miRNA assay included specific RT Primers and TaqMan Probes to quantify mature microRNAs (has-miR-146a). For normalization, hsa-miR-RNU48 (RNU6B) 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 $p<0.05$. The study was approved by the Hospital Ethical Committee and all individuals gave written informed consent in accordance with Declaration of Helsinki.

Table 1: Clinical and Demographic characteristics of MTLE-HS patients

Clinical and demographic characteristics	Patients
Gender (F/M)	6 / 8
Age, years (mean, range)	44.0 ± 10.5 (23 - 65)
Febrile seizures (Yes / No)	7 / 7
Epilepsy age of onset, years (mean, range)	14.4 ± 7.0 (1 - 25)
Hippocampal sclerosis (Left, right, bilateral)	7 / 6 / 1
Refractory to treatment	13

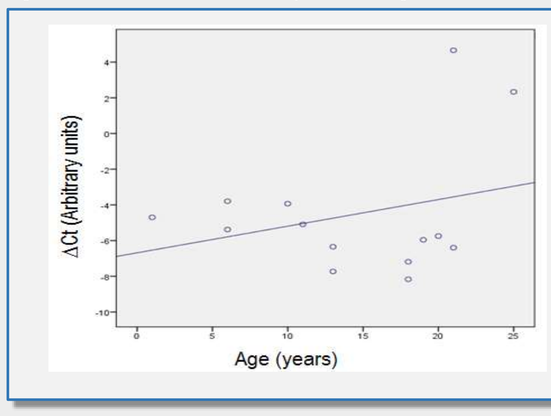
Results

Fig 2 - miR-146a expression in serum



The expression of miR-146a was 2 fold higher in MTLE-HS patients than in controls (Figure 2).

Fig 3 - Correlation between miR-146a expression and age of onset of MTLE-HS



No correlation between MTLE-HS age of onset and miR-146a expression was found (Figure 3).

Conclusion

Studies in animal models and hippocampus from MTLE-HS patients demonstrated that miR-146a is expressed not only in neurons but also in astrocytes during all phases of epileptogenesis (Aronica *et al.* 2010). Our results obtained in serum corroborated these data what may confirm that miR-146a is a suitable biomarker of epileptogenesis. The exact role of miR-146a in epilepsy remais unknown but 2 pathways have been suggested (Figure 4).

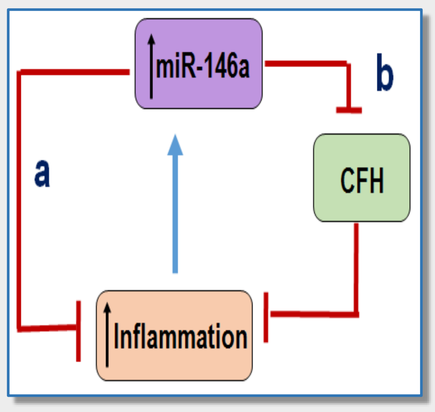


Fig 4 - miR146a and regulation of inflammation

a) Spatio-temporal analysis revealed that miR-146a overexpression is coincident with the upregulation of pro-inflammatory pathways during epileptogenic process. Since miR-146a has a role in fine-tuning the response to cytokines it is thought that its upregulation in epileptic patients may be a compensatory mechanism to overcome the exacerbated inflammatory response (Aronica *et al.* 2010).

b) On the other hand miR-146a expression induces downregulation of CFH (Complement Factor H) an inhibitor of inflammatory pathways, leading to the perpetuation of inflammation (Lukiw *et al.* 2008; Boon *et al.* 2009)

In fact, it has been shown that CFH expression is induced during epileptogenesis (Aronica *et al.* 2007) and may be observed in miR-146a positive glial cells (Aronica *et al.* 2010).

These 2 pathways may represent different cell responses during the several phases of epileptogenic process or may be the reflex of an abnormal function due to changes in the metabolic state of the cells.

Thus, the ultimate effect of miR-146a depends on the availability of other molecules. The comprehension of miR-146a function in epileptogenic mechanism may be relevant for the development of new therapeutic strategies.