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Decreased expression of thyroid receptor-associated protein 220 in temporal lobe tissue of patients with refractory epilepsy

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Abstract

Purpose: TRAP220 (thyroid hormone receptor-associated protein) functions as a coactivator for nuclear receptors and stimulates transcription by recruiting the TRAP mediator complex to hormone responsive promoter regions. Thus, TRAP220 enhances the function of thyroid/steroid hormone receptors such as thyroid hormone and oestrogen receptors. This study investigated the expression of TRAP220 mRNA and protein level in epileptic brains comparing with human control.

Methods: We examined the expression of TRAP220 mRNA and protein levels in temporal lobes from patients with chronic pharmacoresistant epilepsy who have undergone surgery.

Results: Expression of TRAP220 mRNA and protein was shown to be decreased significantly in the temporal cortex of the patients with epilepsy.

Conclusions: Our work showed that a decrease in TRAP220 mRNA and protein levels may be involved in the pathophysiology of epilepsy and may be associated with impairment of the brain caused by frequent seizures.

Keywords: TRAP220; Refractory epilepsy; Thyroid/steroid hormone receptors

In the process of chronic seizure development, many changes in the physiology of the neuroendocrine system can occur. Both epileptic seizures and antiepileptic drugs may alter thyroid/steroid hormone metabolism [1]. Subclinical hypothyroidism or a depressive effect on thyroid hormone induced by some antiepileptic drugs has been observed [2–4]. On the other hand, hormone changes can have an impact on epilepsy. Kalkbrenner's [5] study showed the seizure frequency of rats which were ovariectomized was more than that of non-ovariectomized female rats and rats who accepted estrogen treatment after ovariectomization. The damage of hippocampal neurons caused by seizures was also more severe in rats who were ovariectomized. Vitamin D receptors also appear to be related with epilepsy susceptibility. Potential latency before onset was shortened significantly in VDR gene knockout mice [6].

Nuclear receptors play a critical role for the physiologic function of the thyroid/steroid hormone. The nuclear receptor superfamily comprise a large group of transcriptional regulators which regulate transcription of target genes by binding to specific DNA sequences called hormone response elements in a ligand-dependent manner [7]. Multiple transcriptional coactivators have been identified that bridge nuclear receptors to chromatin and the components of the basal transcription machinery [8,9].

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TRAP220 is an important coactivator and contains two signature L*XX*LL motifs (LXM1 and LXM2), which are required for interaction with the ligand-binding domain of nuclear receptors [10,11]. TPAP220 takes part in a wide range of physiological processes in vivo by anchoring other TRAP subunits to the conserved AF-2 domain—the L*XX*LL core motif. It interacts with ligand-activated nuclear receptors including thyroid hormone receptor, oestrogen receptors, and vitamin D receptor [12]. TRAP220 may have an important function in the brain. A recent study showed TRAP220 mRNA expression in different layers of cerebral neocortex during development of the central nervous system and also in some regions of the adult brain rodents [13]. TRAP220 may have function in adult brains indirectly by interaction with nuclear receptors such as thyroid and oestrogen receptors, which are important for the brain.

In a prior study, in cDNA microarray examination we found TRAP220 decreased by five times in epileptic brains compared to the control. In order to investigate expression of TRAP220 mRNA and protein level, we used several methods including FQ rt-PCR, Western blot, immunohistochemistry and immunofluorescence.

Materials and methods

Subjects. The 40 cases with refractory epilepsy included in this study were obtained from the files of the Departments of Neurosurgery of the following hospitals: the First Affiliated Hospital of Chongqing University of Medical Sciences, Beijing Tiantan Hospital, Xuanwu Hospital of the Capital University of Medical Sciences, and Xinqiao Hospital of the Third Military Medical University. Patients underwent resection of temporal lobe tissue. Before surgery, informed consent was obtained for the use of human brain tissue for research, and the study was approved by the local Ethic Committee. Two neuropathologists reviewed all the cases independently. The diagnosis of seizure type was confirmed according to the 1981 International Classification of Epileptic Seizures of the International League Against Epilepsy.

Before surgery, the epileptic lesion was localized in all patients by brain magnetic resonance imaging (MRI) or CT and 24 h EEG or Video-EEG; Sphenoidal electrode monitoring and intraoperative electrocorticography (ECOG) were performed to localize the epileptic lesion before resection in all patients.

The type of seizure patterns included complex partial seizures, secondary seizures and generalized tonic-clonic seizures. All patients were refractory to maximal doses of at least three or more AEDs including Phenytoin (PHT), Valproic acid (VPA), Carbamazepine (CBZ) and Phenobabital (PB), Topiramate(TPM), Lamotrigine(LTG). Table 1 summarizes the clinical features as follows.

After lesion resection, the electrodes of ECOG were placed on the remaining edge of tissue to ensure the lesion was resected completely. ECOG did not show spikes in nine cases and they underwent anterior temporal lobe resection according to the result of other examinations before surgery. Subpial alba fibers were also transected in five cases except for anterior temporal lobe resection where ECOG still found spikes after brain tissue resection.

The 14 controls were obtained from the files of the neurosurgery department of the first affiliated hospital of Chongqing University of Medical Sciences. These samples comprised of temporal neocortical tissue adjacent of lesion. All patients were diagnosed by pathology as brain trauma. The two neuropathologists also reviewed these cases, which had no history of seizures or other neurologic disorder. Table 2 shows the clinical features of the control.

Tissue preparation. One part of resected brain tissue was immediately placed in cryovial which had been soaked in buffered DEPC(1:1000) for 24 h and then stored in liquid nitrogen, which was used for mRNA analysis and Western blot. Other parts were fixed in 10% buffered formalin. After being fixed in Formalin for 24–48 h, paraffin embedded tissue was sectioned at 4 μm and then mounted on polylysine-coated slides for immunohistochemistry analysis. One section of every specimen was processed for hematoxylin–eosin stains. The same preparation process was performed for analysis of tissue from control.

RNA extraction. Total RNA was isolated from dissected brain tissues, Isolated RNA samples were dissolved in RNase-free Milli-Q liquid. The integrity of samples of the two groups were examined by running 3 μg of the total amount to electrophoresis on a 1% agarose gel and then stained with ethidium bromide to determine the relative intensities of the 28s, 18s and 5.8s rRNA bands. The optical densities (OD value) were measured at 260 nm (A_{260}) and 280 nm (A_{280}) wavelengths and A_{260}/A_{280} ratios were calculated. When A_{260}/A_{280} ratios range between 1.7 and 2.2 the RNA samples was prepared for cDNA microarray and FQ rt-PCR. All RNA samples were stored at −80 °C.

cDNA array. Samples from the experimental ($n = 40$) and control group ($n = 14$) were pooled for cDNA array. The cDNA microarray (BiostarH-40s, Biostar genechip Inc, Shanghai) comprised 4096 elements including human full-length genes. The cDNA probes were generated by RNA reverse transcription under the reverse transcriptase system of RNase-free (Biostar, Shanghai). The array membrane was denatured at 95 °C for 2 min and the slide samples for 30 s. Prehybridization reaction was performed at 42 °C for 5–6 h. Probe labeling was performed during reverse transcription by incorporating cy5dCTP or cy3dCP in a light-lock chamber according to the instruction manual. Subsequently, each group was hybridized in the same solution at 42 °C for 16–18 h. Membranes were washed at 60 °C for 20 min. Membranes were then dried and scanned (ScanArray4000, Biostar, Shanghai). Images of the DNA array blots for the identification of differentially expressed genes was conducted by GenePixPro3.0 software. The intensity value of the genes were normalized using the expression levels of the housekeeping genes spotted on the array. Genes in epileptic tissue were considered to be overexpressed if intensity value was increased two folds compared to the control group(Cy5/Cy3 > 2). Conversely, gene expression was considered down-expressed when Cy5/Cy3 < 0.5.

FQ rt-PCR. The same sample pool was used in FQ rt-PCR. Real-time quantitative PCR was performed according to the manufacturer's protocol. Samples of each group was treated and by DNase 5 μg RNA was conducted as a reverse transcription template. The primer was synthesized for use in FQ rt-PCR: forward 5′ GCTTGTGCGTCAAGTCATGG 3′, reverse 5′ CGTGATGTAACATTCAGTGC 3′. The PCR mixture was diluted 10 times as template, pre-experiment was performed using HS Ex taq DNA polymerase in PCR equipment, whose conditions included 2 min at 94 °C, 30 s at 94 °C, 30 s at 61 °C, 20 s at 72 °C, and 5 min at 20 °C for 30 cycles. The results were determined by agarose gel electrophoresis and choice samples without mixed band for further reaction. PCR amplification was performed using SYBR Premix Ex Taq kit (Takara Inc) in Rotor-Gene RG-3000 Real-Time Thermal Cycler (Corbet Research, Australia) with 18s rRNA as housekeeping gene. The thermal cycling conditions of 40 cycles of amplification included 10 s at 94 °C, 5 s at 95 °C and 30 s at 60 °C. A fluorescence threshold was chosen to determine the first cycle (C_T) where the signals were above the threshold value. The experiments were repeated three times in duplicate. Rotor-Gene 5.0 and Excel 7.0 were used to analyze the fluorescence result in real-time PCR.

Western blot. Western blot analysis was performed to compare the TRAP220 immunoreactive protein levels in refractory epilepsy and the control group according to the manufacturer's protocol. The samples were chosen randomly from epileptic tissue ($n = 4$) and control ($n = 3$) and were cut into small pieces and homogenized in Buffer including protease inhibitors (15 μg/ml aprotinin, and 1 mM phenylmethylsulfonyl fluoride), then centrifuged at 16.000*g* at 4 °C for 5 min and then the crude nuclear pellet was lysed in a similar buffer for 30 min and centrifuged at 16,000*g* at 4 °C for 15 min. The protein concentration of the lysates was determined by Coomassie blue G-250 kit (Sigma, USA). The nuclear extracts (40 μg)