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Increased expression of annexin A7 in temporal lobe tissue of patients with refractory epilepsy

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Summary. Annexin A7 is a member of the family of annexins, which are thought to function in the regulation of calcium homeostasis and the fusion of vesicles. Refractory epilepsy may be related to the imbalance of calcium homeostasis. Our aims are to investigate the expression of Annexin A7 in epileptic brains in comparison with human controls and to explore Annexin A7's possible role in refractory epilepsy. We examined the expression of Annexin A7 via immunohistochemistry, double-label immuno-fluorescence and western blot. The expression of Annexin A7 was shown to be significantly increased in patients with refractory epilepsy. Double-label immunofluorescence and confocal microscopy disclosed Annexin A7 immunoreactivity in the neurons, which were recognized by the antibody of neuron specific enolase (NSE). The result showed that Annexin A7 may be involved in the pathophysiology of refractory epilepsy and may play a role in developing and maintaining the epilepsy.

Key words: Annexin A7, Refractory epilepsy, Calcium homeostasis, Pathogenetic mechanisms, Human brain

Introduction

Epilepsy is a chronic neurological disorder characterized by recurrent spontaneous seizures that are caused by sudden aberrant neuronal discharge. Approximately 1% of the world's population suffers from epilepsy, which numbers around 9 million patients in China. It is estimated that approximately 20-30% of epileptic patients do not achieve adequate seizure control with the use of antiepileptic drugs (AEDs). This type of epilepsy is referred to as drug-resistant epilepsy (Regesta and Tanganelli, 1999). The pathogenetic mechanisms underlying epileptic seizures are still poorly understood. The mechanisms that have been associated with seizure recurrence include the imbalance of calcium homeostasis (DeLorenzo et al., 2005), multi-drug resistance protein over-expression (Kwan and Brodie, 2005; Chengyun et al., 2006), axonal sprouting and neurogenesis (Zucchini et al., 2005) and the activity of reactive astrocytes (Jabs et al., 2008; Wetherington et al., 2008).

Ca^{2+} signaling has a pivotal role in the regulation of many cellular processes. The regulation of intracellular calcium homeostasis is very complex and includes many proteins, such as Ca^{2+} channel proteins (Gargus, 2009), IP3R (Matsumoto and Nagata, 1999; Missiaen et al., 2000), RYR (Sutko and Airey, 1996; Missiaen et al., 2000) and Anxa7 (Döring et al., 1995; Srivastava et al., 1999; Herr et al., 2001; Clemen et al., 2003; Watson et al., 2004; Schrickel et al., 2007). Previous research has explored the relevance of some of these proteins to refractory epilepsy; however, there is little evidence or information on the role of Anxa7.

Annexin A7 (Anxa7, also called synexin) is a member of the class of Ca^{2+}- and phospholipid-binding proteins that are characterized by a bipartite structure comprised of a variable N-terminal domain and a conserved C-terminal domain of Ca^{2+}- and phospholipid-binding sites. The unique N-terminal domains are thought to confer functional diversity (Gerke and Moss, 2002). Anxa7, which was the first of this class of proteins to be discovered, is an agent that mediates the aggregation of chromaffin granules and the fusion of membranes and phospholipids in the presence of Ca^{2+}-ions (Creutz et al., 1978). Anxa7 has 47-kDa and 51-kDa isoforms that have different N-terminal domains and exhibit a tissue-specific expression pattern.

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The 47-kDa isoform is present in all tissues except for skeletal muscle, while the 51-kDa isoform is exclusively present in all tissues. Both isoforms are expressed in the heart muscle, brain tissue and red blood cells (Magendzo et al., 1991; Selbert et al., 1995; Clemen et al., 1999; Herr et al., 2003). At present, considerable evidence supports the view that Anxa7 plays a role in regulating intracellular calcium homeostasis (Döring et al., 1995; Srivastava et al., 1999; Herr et al., 2001; Clemen et al., 2003; Watson et al., 2004; Schrickel et al., 2007) and forming a Ca^{2+} channel (Pollard et al., 1992). Since the maintenance of intracellular calcium homeostasis is essential for normal brain function, it is conceivable that Anxa7 is abnormally expressed in brains of refractory epileptic patients whose maintenance of calcium homeostasis is at risk.

To investigate the expression of Anxa7 levels, we used immunohistochemistry, western blots and double-label immunofluorescence for localization.

Materials and methods

Patient selection

In this study, 38 patients with refractory epilepsy with the typical clinical manifestations and characteristic electroencephalograms were selected at random from 240 patients in our epilepsy brain bank. Among the total, males: 24, females: 14, age: 8-48 years, mean age: 25.92±10.39 years, duration: 3-39 years, mean duration: 13.5±7.5 years. the secondary generalized tonic - clonic seizure from partial seizure: 16 cases, generalized tonic - clonic seizure: 8 cases, complex partial seizure:10 cases, multiple seizure type: 2 cases (including simple, complex partial seizure), simple partial seizure: 1 case, secondary epilepsy: 1 case. All the brain tissues were obtained from temporal neocortex. Table 1 summarizes the clinical features as follows.

Before surgery, informed consent was obtained from the patients or their relatives for the use of their data and brain tissues for research. This study was approved by the National Institutes of Health and the Committee on Human Research at Chongqing Medical University.

Pre-surgical assessment included a detailed history, neurological examination, interictal and ictal video-EEG monitoring, neuropsychological testing and neuro-radiological studies. Before surgery, each epileptic lesion was localized by 24-h EEG or video-EEG, brain magnetic resonance imaging (MRI) and intraoperative electrocorticography (ECOG). All patients had taken maximal doses of at least three or more antiepileptic drugs (AEDs), including phenytoin, valproic acid, carbamaze-pine, phenobarbital, topiramate lamotrigine and traditional Chinese medicine. The diagnosis of the seizure type was classified according to the 1981 International Classification of Epilepsy Seizures of the International League Against Epilepsy.

For comparison, 11 samples were obtained from the neurosurgery department of the first affiliated hospital of Chongqing University of Medical Sciences. Among them, males: 6, females: 5, age: 13-31years, mean age: 21.82±6.4years. Table 2 shows the clinical features of the control. These samples contained temporal neocortical tissue adjacent to the trauma lesions. All patients were diagnosed by pathology as serious brain trauma and need to debridement and intracranial decompression in order to save life. No patients had prior history of seizures, exposure to antiepileptic drugs or other neurologic disorders. The two neuropathologists also reviewed these cases.

Tissue Preparation

One part of the resected brain tissue specimen was immediately placed in a cryovial that had been soaked in buffered DEPC (1:1000) for 24 h and conserved in liquid nitrogen. This part of the brain was used for Western blot. Other parts were fixed in 10% buffered formalin for 48 hours, embedded in paraffin and sectioned at either 5 μm for immunohistochemistry or 10 um for immuno-fluorescence analysis. These sections were placed on polylysine-coated slides. The same process was employed for the brain tissue specimens from the controls.

Immunohistochemistry

All paraffin sections were deparaffinized in xylene, rehydrated in graded ethanol, which was perforated by Triton X-100 (0.4%, for 15 min), and incubated in H_2O_2 (3%) for 10 minutes at room temperature to block endogenous peroxidase activity. To retrieve the antigen, the sections were heated in a 10 mmol/l sodium citrate buffer pH6 in a microwave oven for 20 min at 92–98ºC. The sections were then blocked in normal goat serum for 30 minutes at 37°C to reduce non-specific binding (Zhongshan Gold Bridge Inc., Beijing, China). The sections were subsequently incubated in monoclonal mouse anti-human Anxa7 primary antibody (1:150, BD Biosciences, USA) at 4ºC overnight. This was followed by incubation with biotinylated goat anti-mouse secondary antibody for 30 minutes at 37ºC. After washing with PBS, sections were treated with avidin-biotin peroxidase complexes for 30 min at 37ºC (Zhongshan Golden Bridge Inc., Beijing, China). Immunoreactivity was observed with 3, 30-diaminobenzidine (DAB, Zhongshan Gold Bridge Inc., Beijing, China), and subsequent counterstaining was performed with Harris hematoxylin. Negative controls were processed by substituting PBS for the primary antibody. An AX80 microscope (Olympus, Tokyo, Japan) was used for photomicrography, and a TC-FY-2050 pathology system (Yuancheng Inc., Beijing, China) was used for image acquisition. The quantification of the optical densities of every random visual field was assessed with the Motic Med 6.0 CMIAS pathology image analysis system (Beihang Motic Inc., China). Optical density values were expressed as the mean

±standard errors and analyzed by two-sample t-test that followed the normality test. Differences were considered significant at $P<0.05$.

Double-label immunofluorescence and confocal microscopy

Sections were deparaffinized, rehydrated, perforated and antigen recovery was conducted as described earlier. After blocking with normal goat serum (Zhongshan golden bridge Inc., Beijing, China) for 30 min at 37°C, the sections were incubated with a mixture of monoclonal mouse anti-human Anxa7 antibody (1:100) and polyclonal rabbit anti-NSE (1:25, Boster bio-technology co. ltd) at 4°C overnight. This was followed by incubation with fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG (1:100, Zhongshan Golden Bridge Inc., Beijing, China) and tetramethyl-rhodamine isothiocyanate (TRITC) conjugated goat anti-rabbit IgG (1:00, Zhongshan Golden Bridge Inc., Beijing, China) in the dark for 2 h at 37°C. After an extensive wash with PBS for at least 1h, the sections were mounted with a mixture of 50% glycerol and 50% PBS. Fluorescent-stained sections were surveyed by confocal microscopy (Leica Microsystems Heidelberg GmbH, Germany).

Western blotting

Approximately 0.04 g of cerebral cortex was resected from the tissue samples stored in liquid nitrogen, homogenized in cold RIPA Lysis Buffer (Bioteke Corporation, Beijing, China) in a glass tissue grinder and then centrifuged at 12,000 g for 10 min at

Table 1. Clinical features of the 38 patients with refractory epilepsy.

No.	Age	Gender	Duration	Antiepileptic drugs	Seizure type	Resection tissue	Pathology
1	27	F	14	PHT,PB,CBZ,TCM	CPS,GTCS	TL R P	NL
2	29	F	24	VPA,PB,TMP,	GTCS	TL R A	ND,G
3	26	M	4	PHT,VPA,PB ,	SGS	TL L	ND
4	16	F	15	CBZ,PB,PHT	CPS	TL L A	G
5	14	M	7	TPM,OXC,VPA	CPS	TL L A	ND,G
6	13	M	12	VPA,TMP,PB,PHT	CPS,GTCS	TL L	ND,G
7	8	M	5	PB,VPA,TPM,TCM	CPS	TL L A	G
8	15	M	15	TCM,PB,PHT,VPA,TPM	CPS,GTCS	TL L A	G,NL
9	17	F	5	VPA,TPM,PB	SPS	TL R A	G,ND
10	30	M	10	CBZ,VPA,TPM,PB	GTCS	TL R	G,ND
11	23	M	5	TPM,VPA,TCM	SPS,GTCS	TL R	G,NL
12	42	F	4	CBZ,VPA,OXC	CPS	TL L	G
13	32	F	7	PHT,CBZ,VPA	GTCS	TL L A	G,ND,NL
14	18	F	16	VPA,CBZ,PB,PHT	CPS,GTCS	TL R A	G
15	35	M	9	CBZ,PHT,VPA.TCM	GTCS	TL L A	G
16	26	M	9	CBZ,PHT,VPA,CLB	CPS,GTCS	TL L A	ND,G
17	20	M	10	TPM,VPA,PHT,CLB	CPS,GTCS	TL R A	G,ND
18	36	F	16	PHT,PB,CBZ,VPA	GTCS	TL L A	G
19	16	M	14	PHT,PB,CBZ,	CPS,GTCS	TL L A	G,NL
20	22	M	12	CBZ,VPA,TPM,PB	GTCS	TL R	G
21	17	M	5	TPM,VPA,PHT	CPS,GTCS	TL R A	G,ND
22	18	M	13	TPM,PHT,VPA,LTG	GTCS	TL R A	G,ND
23	21	M	10	PHT,CBZ,TPM,PB,CLB	CPS,GTCS	TL L A	G,ND,NL
24	41	M	20	PHT,PB,TPM,PB,TCM	CPS,GTCS	TL R A	ND,G,NL
25	32	F	19	VPA.CBZ.TPM,PB,TCM	CPS	TL L A	G
26	30	M	29	PHT,PB,VPA,TMP	CPS.SPS	TL L	G,NG
27	44	F	20	PHT,VPA,TMP,LTG,CLB	SPS,GTCS	TL R	G
28	33	M	30	VPA.PB,CLB	GTCS	TL L	G,ND,NL
29	17	M	10	VPA,CBZ,PHT,PB	CPS	TL L	G, ND
30	16	F	6	CBZ,VPA,PHT,PB	CPS	TL L A	AC
31	48	F	30	CBZ.PB,PHT,TMP,CLB	CPS,GTCS	TL R A	G,NL,ND
32	24	M	14	VPA,PB,TMP,LTG	CPS	TL R	G,ND
33	41	F	15	VPA,TMP.PB,LTG,CLB	CPS,GTCS	TL L	G,ND,NL
34	18	M	16	PHT,CLB,VPA,TMP	CPS	TL L A	G,ND
35	29	F	20	PHT,VPA,PB,TMP	CPS,SPS	TL R	G,NL,ND
36	16	M	12	VPA,PHT,PB,TMP,LTG	CPS,GTCS	TL L	G,ND
37	28	M	3	CBZ,PHT,PB,VPA.LTG	CPS	TL L	AC
38	47	M	27	CBZ,VPA,CLB,TMP,PHT	CPS,GTCS	TL R	G,NL,ND

M: male, F: female; CBZ: carbamazepine; PB: phenobarbital; PHT: phenytoin; VPA: valproate; TPM: topiramate; LTG, lamotrigine; CLB, clonazepam; TCM: traditional Chinese medicine;CPS: complex partial seizure; SPS: simple partial seizures; GTCS: generalized tonic-clonic seizure; L: left; R: right; A:anterior; P:posterior; TL: temporal lobe; NL: neurons loss; ND: neurons degeneration; G: gliosis; AC: astrocytosis.

Table 2. Clinical features of the 11 patients as the control.

No.	Age	Gender	Etiologic diagnosis	Resection tissue	Adjacent tissue pathology
1	22	F	Trauma	TL L	Normal
2	28	M	Trauma	TL L	Normal
3	19	F	Trauma	TL R	Normal
4	15	F	Trauma	TL L	Normal
5	31	M	Trauma	TL R	Normal
6	26	M	Trauma	TL R	Normal
7	18	M	Trauma	TL R	Normal
8	29	F	Trauma	TL L	Normal
9	25	F	Trauma	TL R	Normal
10	13	M	Trauma	TL L	Normal
11	14	M	Trauma	TL L	Normal

M: male; F: female; TL: temporal lobe; L: left; R: right.

4ºC to remove the remnant. The protein concentration of the lysates was assayed by the bicinchoninin c acid (BCA) Protein Assay Kit (Pierce Biotechnology, Rockford, IL) for which bovine serum albumin is used as a standard. The proteins (100 ug) were subjected to

Table 3. Clinical characteristics of the subjects.

Characteristics	epilepsy (n=38)	Control (n=11)	Results
Age	25.92±10.391	21.82±6.400	t=1.238, p=0.222
Gender			
Male	24 (63.2%)	6 (54.6%)	
Female	14 (36.8%)	5 (45.4%)	X^2=0.267, p=0.606
Location			
Left	22 (57.9%)	6 (54.6%)	
Right	16 (42.1%)	5 (45.4%)	X^2=0.039, p=0.843

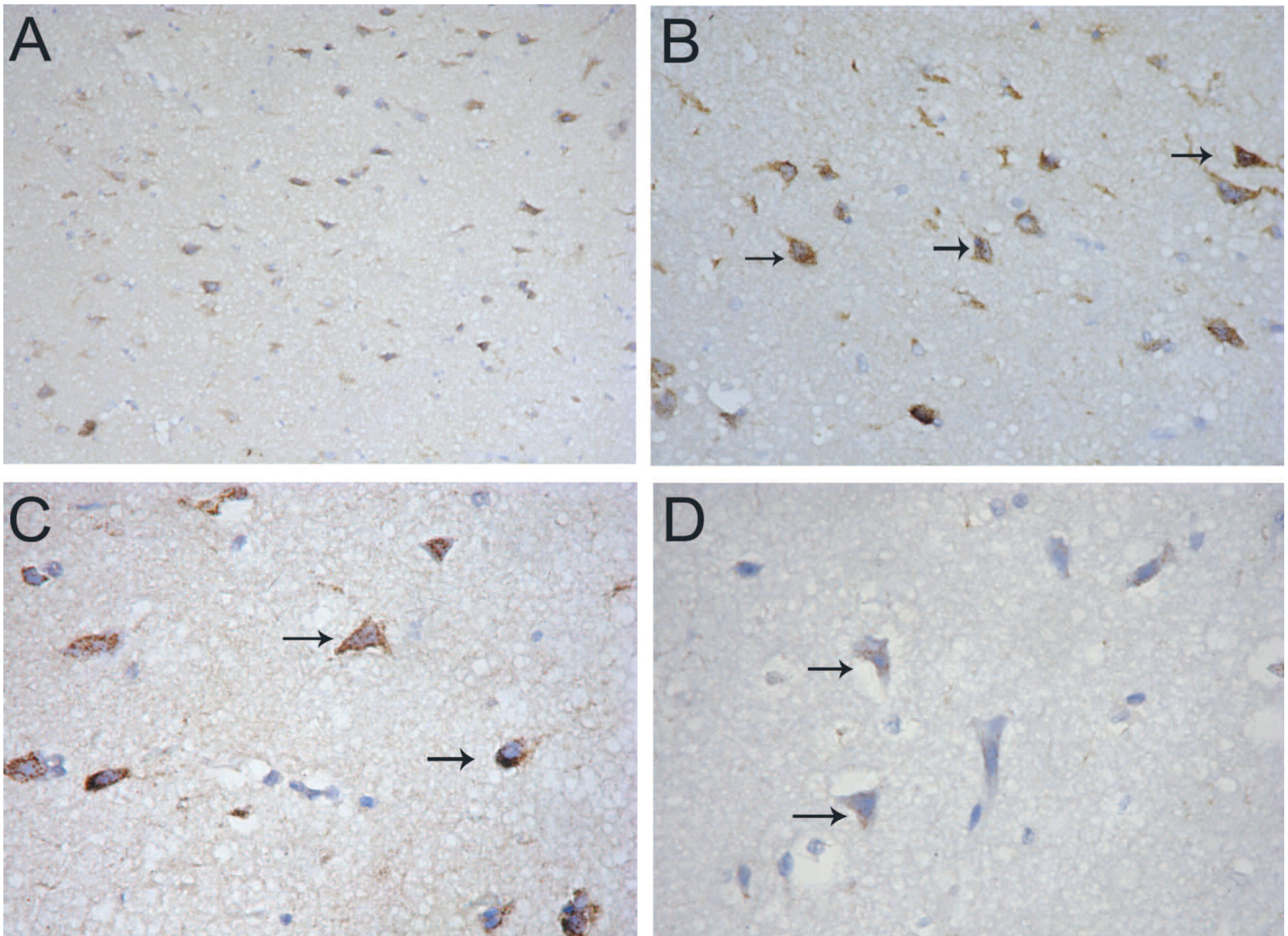

Fig. 1. A-C. Strong positive cells expressed annexin a7 in temporal neocortex of patients with refractory epilepsy. **D.** Scattered annexin a7 positive cells in normal temporal neocortex tissue. The black arrows refer to these annexin a7 positive cells. Scale bars: A, 200 μm; B, 100 μm; C, D, 75 μm.

SDS-PAGE (10%) and electrotransferred to nitrocellulose membranes (Bio-Rad Laboratories, Hercules, CA, USA) at 4°C overnight. The membrane was blocked with TBS containing 10% non-fat dried milk at 37ºC for 1.5 h and incubated overnight at 4°C with the monoclonal mouse anti-human Anxa7 (1:300, BD Biosciences, USA) and rabbit anti-ß-actin (1:350) (Biosynthesis Biotechnology Co., LTD, Beijing, China) immunoglobulin. After washing in TBST, the membranes were incubated with species-specific HRP-conjugated secondary antibodies (1:1500, Zhongshan Golden Bridge Inc., Beijing, China) for 1 h at 37ºC. The immunoreactive bands were visualized using ECL (Pierce Biotechnology, Rockford, IL) and exposed to X-ray film (BioRad Fluor-STM MultiImager). A molecular weight marker (MBI Fermentas, Canada) was loaded in the first channel to determine the size of the positive bands, and the transferred membrane was checked up by Ponceau red. The pixel densities of the images were calculated with Quantity One 4.6 analysis software (Bio-Rad Laboratories). Pixel density data were measured and compared with two-sample *t*-test. Statistical significance was accepted at $P<0.05$.

Results

Comparison of clinical characteristics

Refractory epileptic patients were deemed suitable as candidates for surgery according to their intractability and in concordance with conventional pre-surgical evaluation procedures, such as clinical, neuro-physiological, and qualitative imaging modalities. In our study, 92.1% of patients had a history of seizure recurrence for more than 5 years, and 68.4% of patients had at least a 10-year-long clinical history. The demographic and clinical characteristics of the subjects in the study showed that there was no significant difference in age, gender or focus localization of the studied tissue when compared to the controls (Table 3) ($P>0.05$).

Anxa7 Expression in Temporal Neocortex of Patients with refractory epilepsy

Immunohistochemical staining revealed that the positive Anxa7cells in the temporal lobes of refractory epilepsy cases showed prominent up-regulation (Fig. 1). There was faint and scattered immunoreactivity for Anxa7 positive cells (Fig. 1D), and no observed immunoreactivity in the negative controls since the primary antibody was omitted. The mean optical density (OD) of Anxa7 expression was determined to be 0.3641±0.04197 in epileptic tissue, and 0.1824 ±0.02123 in the controls. Fig. 2 shows the histogram of the optical density value comparisons of the samples' immunohistochemistry. The two-sample t-test shows significant over-expression of Anxa7 in epileptic tissue when compared to the control ($P<0.05$) (Fig. 1).

To further explore the cellular localization of Anxa7, double-label immunofluorescent staining with NSE was performed. NSE is a neuron-specific marker. Anxa7 positive cells were red fluorescent, NSE positive cells were green fluorescent, and the merge image became yellow fluorescent. Thus, the double-label immunofluorescence showed that Anxa7 positive cells coexpressed NSE (Fig. 3).

Western blot

A Western blot was performed to further determine whether the over-expression of Anxa7 in the immunohistochemical staining of the epileptic tissue was a reliable result. Faintly stained bands (two bands in total) were present in all control samples, whereas relatively strong Anxa7 bands (two bands in total) were observed in the epileptic group (Fig. 4). Anxa7 antibody showed double immunoreactive bands with an apparent molecular weight at 47 and 51- kDa. Other bands of ß-actin expression were regarded as a positive control (Fig. 4). The ratio of pixel density value of epileptic patient tissue (two bands, analyzed together) (0.4631±0.26610) was significantly higher ($P<0.05$) than the ratio in control tissue (0.2438±0.14948). This was consistent with our immunohistochemical data. Fig. 5 shows a histogram of the optical density value comparison between both groups.

Discussion

In our study, we found that the expression of Anxa7 was up-regulated in epileptic brain tissues compared to control brain tissue, which suggests that Anxa7 may be involved in the pathophysiology of refractory epilepsy and may play a role in developing and maintaining the epilepsy.

Anxa7, as a member of Ca^{2+}- and phospholipid-binding proteins, has broad expression in the brain. During the study of mice brain development, Anxa7 changed its subcellular localization from the cytoplasm to the nucleus. In adult mice central nervous systems, the subcellular distribution of Anxa7 depends on the cell

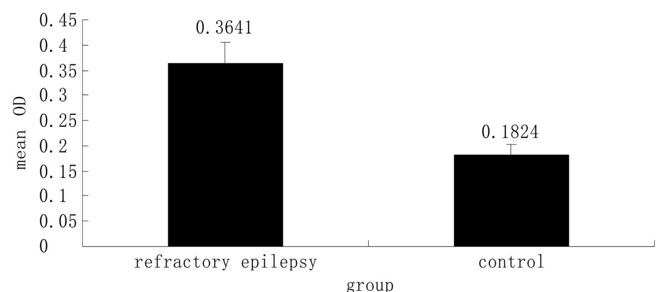

Fig. 2. The quantitative results of mean optical densities (OD) of immunohistochemistry show extensive expression of Anxa7 in patients as compared with control.

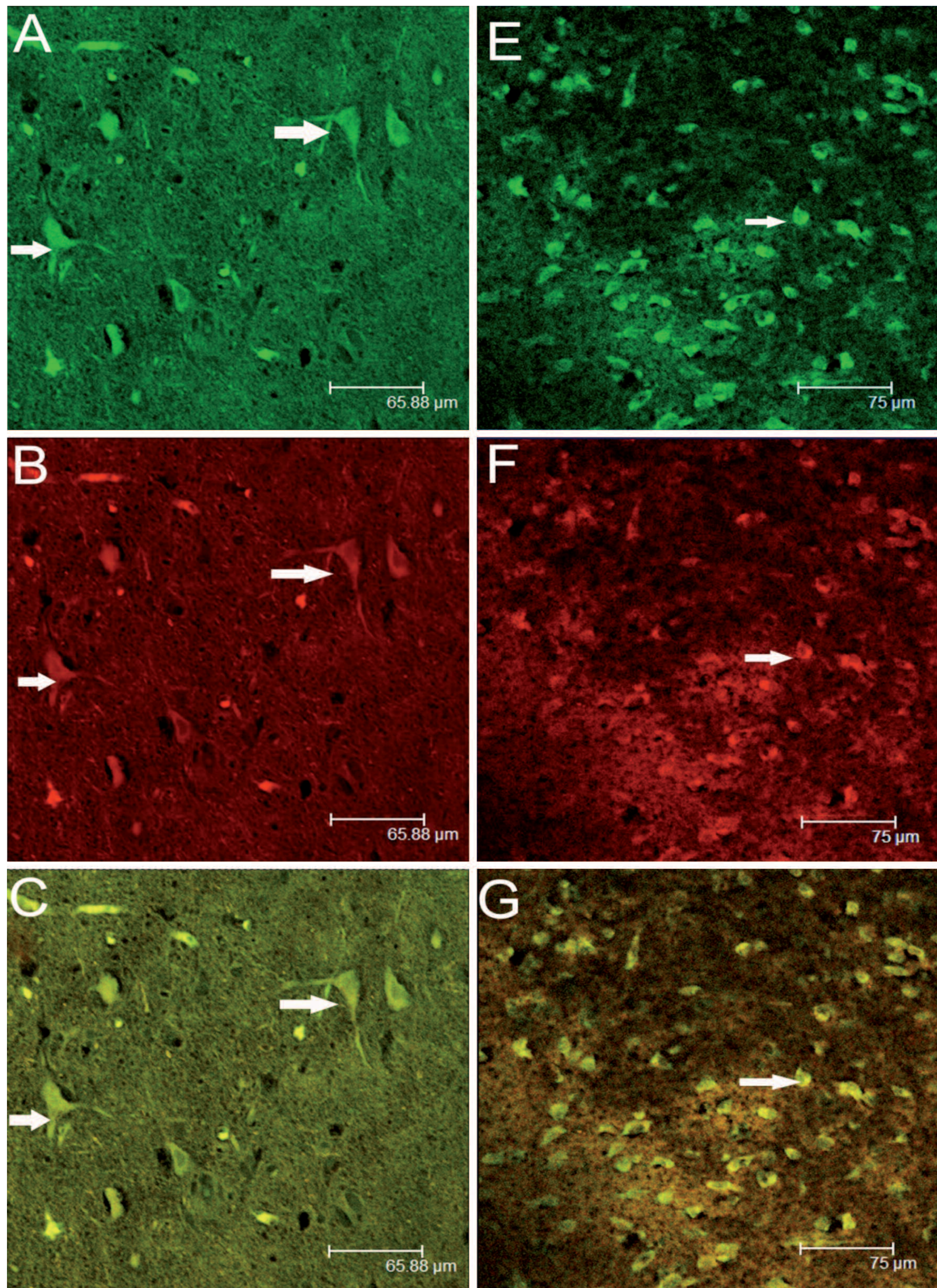

Fig. 3. Double-label immunofluorescence and confocal microscopy to NSE visualized in green (**A, E**), and anxa7 visualized in red (**B, F**) in the temporal neocortex of refractory epilepsy patients. **C and G** showed the merge image.

type. Neuronal cells express Anxa7 in the nucleus, whereas glial fibrillary acidic protein (GFAP)-positive astrocytes exhibited both cytoplasmic and nuclear staining. However, in the adult human isocortex, Pyramidal neurons and apical dendrites exhibit Anxa7 in their cytoplasm, which do not express in their nuclei. Subpial astrocytes exhibited a weak staining of Anxa7 in the cytoplasm (Rick et al., 2005). In our study, Anxa7 was only seen in the cytoplasm of the neurons. The difference in these results may be explained by the fact that they are derived from the parietal neocortex of human autopsy brains, while ours were in the temporal neocortex and were obtained from patients undergoing neurosurgery for therapy-refractory epilepsy. Clemen et al. (2003) found that astrocytes from an Anxa7-/- mouse exhibited a remarkably increased proliferation rate in cultured mutant astrocytes. Most astrocytes in epilepsy belonged to reactive astrocytes (Jabs et al., 2008; Wetherington et al., 2008). So they could proliferate fast due to lack of Anxa7 and form glial scar in the brain, which has some deleterious effects, including inhibition of axonal sprouting in the damaged brain. Thus, they could inhibit drugs from the lesions that would result in the drug-refractory epilepsy (Zhou et al., 2008).

Anxa7 plays an important function in the formation of a Ca^{2+} channel (Pollard and Rojas, 1988; Burns et al., 1989; Pollard et al., 1991, 1992) and regulation of intracellular calcium homeostasis in several cell types

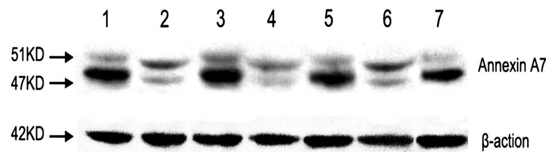

Fig. 4. Western blot: X-ray film of Anxa7 shows that the expression of Anxa7 increasing in reftractory epilepsy. Case 1, 3, 5, 7 indicate the band of refractory epilepsy and case 2, 4, 6 indicate the control tissue. The protein band in epileptic tissue is stronger than the control.

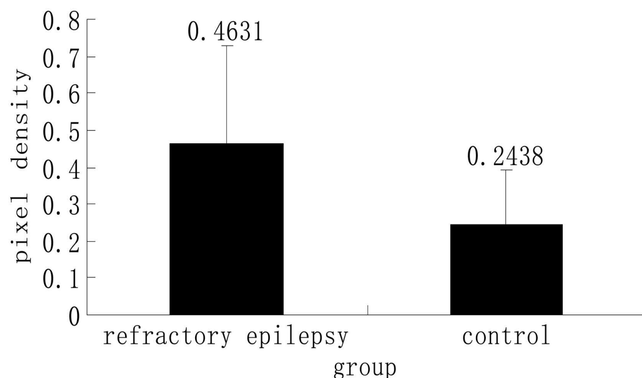

Fig. 5. The statistic results of pixel densities of Western blot.

and species (Döring et al., 1995; Srivastava et al., 1999; Herr et al., 2001; Clemen et al., 2003; Watson et al., 2004; Schrickel et al., 2007). Srivastava et al. (1999) described that an Anxa7 -/-mouse is lethal at embryonic day (E10) because of cerebral hemorrhaging. In addition, Anxa7-/+ mice exhibit an insulin secretion deficit because of defective intracellular pancreatic Ca^{2+} signaling processes with a marked reduction in IP3-sensitive Ca^{2+} (1999). However, the null strain reported by Herr et al. (2001) was viable and healthy and showed no insulin secretion or other obvious defects. Cardiomyocytes from adult mice exhibit an altered cell shortening-frequency relationship when stimulated with high frequencies. The authors suggested that the conflicting results could be due to the altered expression of genes in the vicinity of the integration site or to a different genetic background. In addition, Schrickel et al. (2007) recently reported that Anxa7 deficiency causes severe electrical instability in the murine heart, including conduction disturbances and anisotropy of impulse propagation. Current work by Watson et al. (2004) showed that Anxa7 (+/-) mice have a markedly reduced IP3 sensitivity in brain ER calcium pools. This provided the first evidence for a possible role of Anxa7 in normal function and diseases of the central nervous system. The over-expression of Anxa7 may cause intracellular calcium overload via IP3R mediated Ca^{2+} induced Ca^{2+} release by increasing the sensitivity of IP3. All of this evidence suggests that Anxa7 contributes to the dysregulation of intracellular calcium homeostasis.

The normal maintenance of Ca^{2+} homeostasis is essential to normal neuronal function (DeLorenzo et al., 2005). Cytosolic calcium ions originate either as extracellular calcium that enters the cell through plasma membrane ion channels or from the release of an intracellular store in the endoplasmic reticulum (ER) through ER inositol triphosphate receptor (IP3R) and ryanodine receptor (RyR) channels (Gargus, 2009). The regulation of intracellular calcium homeostasis is very complex and rigorous. The disruption of intracellular calcium homeostasis by excessive Ca^{2+} influx or release from intracellular stores can lead to the inappropriate activation of Ca^{2+}-dependent processes and cause metabolic derangements and eventual cell death (Arundine and Tymianski, 2003). Several studies have led to the development of the Ca^{2+} hypothesis of epileptogenesis that implicates Ca^{2+} as a second messenger involved in the induction and maintenance of epilepsy (DeLorenzo et al., 2005). The over-expression of Anxa7 in the brain tissue of intractable epilepsy can enhance IP3-mediated Ca^{2+} release or increase Ca^{2+} influx through plasma membrane ion channels, which could lead to intracellular calcium overload and disturb intracellular calcium homeostasis. This disturbance could trigger pathological plasticity changes that lead to the development of epilepsy. Ca^{2+} signals can regulate gene transcription through multiple Ca^{2+}-activated enzyme and transcription factor pathways (DeLorenzo et al., 2005). Thus, long-term calcium overload can also

promote over-expression of the Anxa7 gene, which plays a role in maintaining chronic epilepsy.

Epilepsy causes over-expression of Anxa7, while Anxa7 takes part in the progress of epilepsy and promotes the further development of epilepsy thereby forming a vicious circle. This is obviously just our hypothesis, and more work needs to be done to understand the mechanism of Anxa7 in epilepsy. Of particular importance is the question of why the 47-kDa isoform increases so significantly in epilepsy patients, while the controls have a relative increase of the 51-kDa isoform. Answering this question would require an in-depth knowledge of the functions of the two isoforms. In our study, abnormal expression of Anxa7 can be seen in temporal lobe brain tissue of intractable epilepsy, which localizes in the neuronal cytoplasm. Our study provides a new direction in revealing the mechanisms of epilepsy, especially of the refractory type.

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Conflict of interest statement. None.

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