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Elevated expression of human papillomavirus antigen in brain tissue of patients with Rasmussen's encephalitis

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ABSTRACT

Objects: To investigate the expression of human papillomavirus (HPV)-specific antigen in the brain tissue of patients with Rasmussen's Encephalitis (RE) and its possible link to the clinical manifestation of RE.
Methods: The correlation between RE and HPV antigen expression in brain tissue sections was investigated using immunohistochemical (IHC) staining, pathological examination, MRI and clinical manifestations.
Results: HPV antigen expression was elevated in three out of four patients with RE, whereas there were no detectable HPV antigens in six control patients. Significant staining for HPV antigen was located mainly around or in the nucleus and cytoplasm of neurons. Among these RE patients, three with elevated expression of HPV antigens had obvious hemisphere atrophy, whereas the patient with negative staining for HPV antigens had mild atrophy.
Conclusions: Elevated expression of HPV antigens was observed in the brain tissue of RE patients, which may correlate with hemisphere atrophy. Thus, our results may suggest that HPV infection or being a carrier of HPV may play a role in the initiation and progression of RE.

1. Introduction

Rasmussen's encephalitis (RE), which was first described by Rasmussen in 1958, is a rare, dispersed, and progressive neurological syndrome. It is characterized by unilateral inflammation of the cerebral cortex, focal epilepsy, progressive hemiplegia and cognitive deterioration (Rasmussen et al., 1958). Nearly 50% of patients develop epilepsia partialis continua (EPC) within 1 year after the onset of seizures. Anti-epilepsy drugs have little effect on focal seizures, and anti-inflammatory therapies, including pulse therapy with steroids or immune globulins, are only beneficial for partial RE patients over a short period. Hemispherectomy or any type of surgery disconnecting the affected hemisphere remains the only effective treatment for RE patients with intractable epilepsy (Varadkar et al., 2014).

The etiology and pathogenesis of RE remain unknown. It has been widely accepted that inflammation of hemispherical cortex caused by cytotoxicity of CD8+ T-lymphocytes plays an important role in the initiation and progression of the disease (Bauer et al., 2002; Bien et al., 2002). However, the factors involved in triggering this inflammation process are not clear (Varadkar et al., 2014). The histopathological changes in the brains of RE patients share similar features with viral encephalitis, including lymphocyte infiltration, neuron loss, vascular cuffing, and microgliosis (Varadkar et al., 2014), suggesting that viral infections may act as an etiology of the syndrome. Several research groups have made much effort in detecting virus-specific components. Specific antigens and nucleic acids of human cytomegalovirus (HCMV) and Epstein-Barr virus (EBV) were detected in the brain samples of RE patients, but no group has been able to isolate the live virus (Walter and Renella, 1989; Power et al., 1990; Jay et al., 1995). Schwab et al. reported that the main infiltrating T-lymphocyte population in RE was likely expanded from a few precursor T-cells that responded to discrete antigenic epitopes, which could originate from virus particles (Schwab et al., 2009). Another experiment showed that the

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Table 1
Diagnostic criteria for RE.

RE can be diagnosed if either all three criteria of Part A or two out of three criteria of Part B are present. Check first for the features of Part A. Then, if these are not fulfilled, of Part B. In addition: If no biopsy is performed, MRI with administration of gadolinium and cranial CT needs to be performed to document the absence of gadolinium enhancement and calcifications to exclude the differential diagnosis of a unihemispheric vasculitis.
Part A:
1. Clinical Focal seizures (with or without Epilepsia partialis continua) and Unilateral cortical deficit(s)
2. EEG Unihemispheric slowing with or without epileptiform activity and Unilateral seizure onset
3. MRI Unihemispheric focal cortical atrophy and at least one of the following:
Grey or white matter T2/FLAIR hyperintense signal
Hyperintense signal or atrophy of the ipsilateral caudate head
Part B:
1. Clinical Epilepsia partialis continua or Progressive[a] unilateral cortical deficit(s)
2. MRI Progressive[a] unihemispheric focal cortical atrophy
3. Histopathology T cell dominated encephalitis with activated microglial cells (typically, but not necessarily forming nodules) and reactive astrogliosis. Numerous parenchymal macrophages, B cells or plasma cells or viral inclusion bodies exclude the diagnosis of RE.

[a] 'Progressive' means that at least two sequential clinical examinations or MRI studies are required to meet the respective criteria. To indicate clinical progression, each of these examinations must document a neurological deficit, and this must increase over time. To indicate progressive hemisphere atrophy, each of these MRIs must show hemisphere atrophy, and this must increase over time.

lymphocytes of neonatal mice could eliminate an attenuated lymphocytic choriomeningitis virus (LCMV) from most tissues, except for neurons in central nervous system, where the virus persisted (known as a "predisposing virus"). It was considered that memory T-cells were neither deleted nor sufficiently primed to cause disease; however, they might have been triggered in adulthood after a secondary infection of LCMV (Merkler et al., 2006). Moreover, according to different clinical series, 30%–40% of RE patients showed clinical signs related to viral infections, such as fever, headache and cough a few weeks before seizure onset (Takahashi et al., 2006; Guan et al., 2014). These observations may infer that a viral infection is a potential factor for the occurrence of RE.

Human papillomavirus (HPV) is a type of DNA virus that usually invades epithelial cells. It has been proven that infection by some types of HPV is closely associated with cervical cancer (Faridi et al., 2011). In addition to HCMV and EBV, HPV has been proposed to be correlated with focal cortical dysplasia (FCD), which is the most common etiology for childhood focal epilepsy(Chen et al., 2012). As Wheeler et al. reported (Wheeler et al., 2013), the prevalence for any of HPV types was 52% at women aged from 20 to 30 years. Base on this situation, we speculated that early stage latent HPV infection might serve as a trigger for inflammation and immune attacks in RE patients. Therefore, we investigated the expression levels of HPV antigens in the brain samples of four RE patients, with samples from six non-RE patients served as controls, and analyzed the possible linkage between HPV expression and the occurrence of RE.

2. Materials and methods

2.1. Patients and treatment

Retrospective clinical cohort of consecutive surgical patients with RE (n = 4) who underwent operation in Beijing Sanbo Brain Hospital, Capital Medical University between 1st January 2009 to 31st December 2009 were included in this study. All patients met the 2005 European diagnostic criteria for RE (Table 1). In this study, RE cases were compared with non-RE cases underwent surgery due to intractable epilepsy (n = 6) matched for age at the time of surgery and seizure onset. The clinical evaluation included a medical history and neurological examinations, as well as ictal and interictal scalp video EEG recordings. Neuroimaging studies included CT scans and 1.5 T MRI. Clinical data were abstracted from the medical records including the followings: preceding event, age at seizure onset, gender, age at surgery, interictal and ictal EEG pattern, type of operation, side of operation, seizure outcome after surgery, and duration of follow-up. This study was approved by the Ethics Committee of Beijing Sanbo Brain Hospital, Capital Medical University.

2.2. Brain tissue preparation

According to the MRI results, we categorized brain samples as 'nidus' (high signal in FLAIR) or 'non-nidus' (normal signal in FLAIR). Brain tissues in different brain areas that included 'nidus' and 'non-nidus' regions were collected before hemispherectomy. Brain tissues of non-RE patients were collected at the edge of resected epileptogenic area. The tissues were fixed in 4% paraformaldehyde and embedded in paraffin for sectioning. The paraffin specimens were 4 μm thick. One section was obtained for every 10 sections, and a total of three sections were obtained from each patient. During sectioning, the blade was kept clean and sharp to avoid cracking or detachment of the sections.

2.3. Immunohistochemical staining (IHC)

The tissue sections (4 μm thick) were attached to APES-treated coverslips and were baked at 60° centigrade in an oven for 60 min to melt the paraffin. The sections were immersed in xylene to completely remove paraffin from the surface. The sections were then immersed in a gradient of ethanol to remove the xylene and hydrate the sections. Endogenous peroxidase was inactivated by adding 3% H2O2 in H2O. After antigen retrieval, bovine serum albumin (BSA) in phosphate buffered saline (PBS) with 0.05% Tween 20, was added for blocking. Next, the blocking solution was removed, and the primary antibody (anti-HPV 1, 6, 16, 18, and 31) was added and incubated at 4° centigrade overnight. To prevent non-specific staining, the sections were then washed 3 times with PBS to remove any residue reagents, such as the primary antibody. The secondary antibody was then added, and DAB was used for development. The sections were counterstained with hematoxylin to visualize the outline of the cells. Acid–alcohol was added for bleaching. The hematoxylin in the cytoplasm was washed out, the sections were washed in tap water, and the nuclei were stained blue. The sections were dehydrated in gradient ethanol, cleared in xylene, and mounted using neutral balsam. The sections were observed under a light microscope, recorded, and photographed. A yellow-brown colored reaction in the cells was defined as positive.

2.4. Image acquisition and experimental data processing

The IHC results were evaluated by two specialists, SC and YW, who were unknown to the group, using a previously described scoring methodology (Allred et al., 1998). Positive cells were counted using image analysis software (Image-Pro® Plus 6.0; Media Cybernetics Inc.). Cells showing yellow or brown particles in the cytoplasm or nucleus were considered positive. We firstly selected a high-power field at central area of the brain section, and then moved a randomly distance without staring at the microscope along four directions "upper left", "upper right", "lower left", "lower right" to find four random high-power fields. 100 cells were counted per field. The semi-quantitative results were expressed as the percentage of positive cells combined with a subjective assessment of staining intensity. The staining intensity was scored as 0 (colorless), 1 (light yellow), 2 (yellow or brown), or 3 (dark brown); the percentages of positive cells were denoted as 0 (<5%), 1(5–25%),