EPILEPSY CURRENTS

Current Literature
In Basic Science

Is Focal Cortical Dysplasia an Infectious Disease?

Detection of Human Papillomavirus in Human Focal Cortical Dysplasia Type IIB.

Chen J, Tsai V, Parker WE, Aronica E, Baybis M, Crino PB. Ann Neurol 2012;72:881–892.

OBJECTIVE: Focal cortical dysplasia type IIB (FCDIIB) is a sporadic developmental malformation of the cerebral cortex highly associated with pediatric epilepsy. Balloon cells (BCs) in FCDIIB exhibit constitutive activation of the mammalian target of rapamycin complex 1 (mTORC1) signaling pathway. Recently, the high-risk human papillomavirus type 16 oncoprotein E6 was identified as a potent activator of mTORC1 signaling. Here, we test the hypothesis that HPV16 E6 is present in human FCDIIB specimens. METHODS: HPV16 E6 protein expression was assayed by immunohistochemistry in FCDIIB specimens (n = 50) and control brain specimens (n = 36). HPV16 E6 DNA was assayed by polymerase chain reaction (PCR) and in situ hybridization; HPV16 E6 mRNA was assayed by reverse transcriptase PCR. HPV16 E6 was transfected into fetal mouse brains by in utero electroporation to test the effects of E6 on cortical development. RESULTS: HPV16 E6 protein was robustly expressed in all FCDIIB specimens in BCs, but not in regions without BCs or in control tissue specimens including normal brain, lymphoblasts, and fibroblasts, cortical tubers, and U87 glioma cells. E6 expression in FCDIIB colocalized with phosphoactivated S6 protein, a known mTORC1 substrate. HPV16 E6 DNA and mRNA were detected in representative specimens of FCDIIB but not control cortex, and were confirmed by sequencing. Transfection of E6 into fetal mouse brains caused a focal cortical malformation in association with enhanced mTORC1 signaling. INTERPRETATION: Our results indicate a new association between HPV16 E6 and FCDIIB and demonstrate for the first time HPV16 E6 in the human brain. We propose a novel etiology for FCDIIB based on HPV16 E6 expression during fetal brain development.

Commentary

Focal cortical dysplasia (FCD) is a common malformation of cortical development and an important cause of medically refractory epilepsy. Advances in neuroimaging have led to increasing identification of FCD as the etiology of epilepsy, accounting for up to 25% of cases of focal epilepsy (1). Furthermore, the true prevalence of FCD may be underestimated, as pathological diagnoses of FCD are sometimes made retrospectively in pathological specimens resected from patients previously considered to have nonlesional (i.e., MRI-negative) epilepsy. From a therapeutic standpoint, epilepsy due to FCD is often refractory to available antiseizure medications. Surgical removal of FCD results in seizure freedom in about 50–70% of patients (2, 3), but a significant proportion of epilepsy patients with FCD continue to have seizures despite available medical and surgical options. Development of novel, more effective therapies for epilepsy related to FCD would be aided by a better understanding of the pathogenesis of these developmental brain lesions.

Several types of FCD have been described, based on distinctive pathologic features (4). FCD type II represents an isolated lesion characterized primarily by cortical dyslamination and dysmorphic neurons. The additional pathological hallmark of the subtype, FCD type IIb, is the balloon cell, an enlarged, multinucleated spherical cell with immature glial and neuronal features. Accordingly, the developmental origin of the balloon cell has been hypothesized to be derived from glial or neuronal progenitor cells. However, the specific pathogenic trigger for FCD has remained elusive.

Abnormal signaling of the mammalian target of rapamycin complex 1 (mTORC1) pathway has been implicated in the pathophysiology of FCD and other related malformations of cortical development. In fact, Crino has proposed that FCD is part of a spectrum of cortical malformations characterized by abnormal cortical architecture, cytomegalic cells, intractable epilepsy, and excessive mTORC1 activation, including cortical tubers of tuberous sclerosis complex (TSC) and hemimegalencephaly (5, 6). mTORC1 is an essential cell signaling pathway that, in addition to a number of other important metabolic and physiological functions, stimulates cell growth and proliferation. Balloon cells in FCD and hemimegalencephaly and giant cells in tubers exhibit biochemical markers indicating excessive mTORC1 pathway activation, which could promote cytomegaly.

Assuming mTORC1 is the signaling mechanism driving the formation of balloon cells and other pathological features of FCD, what initially stimulates abnormal mTORC1 signaling? In the genetic disease, TSC, the *TSC1* and *TSC2* genes have a direct molecular link with the mTORC1 pathway. Normally,

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the *TSC1* and *TSC2* gene products inhibit mTORC1 activity and limit cell growth and proliferation; mutation of one of these genes leads to disinhibition or hyperactivation of the mTORC1 pathway, which can cause cytomegaly and increased cell proliferation, hence promoting tuber formation and the associated phenotype of TSC. A similar genetic etiology has also been considered and investigated for isolated FCD (7). To this point, however, only benign genetic polymorphisms in mTORC1 regulators have been identified, but no pathogenic mutations have been definitively established in FCD.

In contrast to putative genetic etiologies, an environmental insult or injury during fetal brain development represents a possible alternative pathogenic mechanism for FCD. In particular, an infectious etiology could be a potential trigger for cortical malformations, but no infectious agent has ever been implicated in FCD. The human papillomavirus type 16 (HPV16) is a common cause of cervical cancer, as well as some oropharyngeal cancers. HPV16 induces cytopathologic changes in cervical epithelial cells, including enlarged cells with multilobulated nuclei similar to balloon cells of FCD. Recent studies have found that the HPV16 E6 oncoprotein activates mTORC1 signaling, indicating a potential biochemical basis for the cytomegalic pathologic findings of cervical cancer (8–10).

Based on this association between HPV16, cytomegaly, and mTORC1 signaling, Crino and colleagues searched for evidence of HPV16 infection in pathological specimens of FCDIIB, using multiple assays for HPV16-specific DNA, RNA, and proteins. Remarkably, they found definitive evidence for HPV16 in all of 50 samples of FCDIIB resected from a cohort of patients with intractable epilepsy. In contrast, no sign of HPV16 was found in any of 36 control specimens, including FCD type IIA and tubers of TSC. Furthermore, expression of HPV16 E6 protein was found primarily within balloon cells and colocalized with markers of mTORC1 pathway activation.

While this 100% association of HPV16 infection with FCDIIB is indeed impressive, it is only a correlation—not proof of causation. Thus, Crino's group took their study one step further, testing whether HPV16 infection is sufficient to cause focal cortical malformations in an animal model. The HPV16 E6 protein was transfected into fetal mouse brain, which led to the subsequent development of a focal cortical malformation. Most E6 transfected cells failed to reach the appropriate cortical layer and accumulated in the subcortical white matter and subventricular zone. Furthermore, E6 infected cells also expressed markers of mTORC1 activation, although, somewhat surprisingly, they were not cytomegalic.

This work is extremely novel and potentially paradigm-shifting for the field of cortical malformations and epilepsy. The general concept of the detrimental effects of prenatal infections on brain development has previously been established, as exemplified by the classic TORCH syndromes. Prenatal TORCH infections can be associated with extensive cortical malformations, such as lissencephaly, pachygyria, polymicrogyria, and schizencephaly (11). However, the possibility that a more localized cortical malformation, such as FCD, could be triggered by an infectious agent has never been demonstrated. As the prevalence of both symptomatic and subclinical HPV infection in women is relatively high, vertical transmission of HPV16 from the mother to the fetus during pregnancy seems feasible but remains to be proven.

Even if HPV16 is the etiological agent for FCDIIB, a number of questions remain to be answered. HPV16 may cause some of the pathological features of FCDIIB, such as the balloon cells, but whether it is responsible for all the components of FCDIIB is not clear. Even in the animal model experiments, HPV16 E6 protein apparently did not cause the formation of cytomegalic, balloon cells. Furthermore, whether HPV16 infection during cortical development actually leads to epilepsy remains to be tested. The pathogenesis of FCD lesion formation and the subsequent development of epilepsy could, in theory, involve separate mechanisms. Nevertheless, the link between HPV16 and FCDIIB potentially opens up completely new avenues of investigation and approaches for intractable epilepsy. While patients with epilepsy due to FCDIIB will likely continue to be treated with standard medical and surgical approaches, these new findings suggest the potential utility of other therapeutic strategies to prevent FCD, such as use of mTOR inhibitors or antiviral agents in high-risk patients, as well as vaccination against HPV16. From a broader clinical and epidemiological standpoint, it may now be appropriate to conceptualize and classify FCDIIB as an infectious disease.

by Michael Wong, MD, PhD

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