Is Focal Cortical Dysplasia an Infectious Disease?

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


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 constitutively active mTORC1 signaling. Recently, the high-risk human papillomavirus type 16 (HPV16) E6 oncoprotein 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, and HPV16 E6 mRNA was assayed by reverse transcriptase PCR. RESULTS: HPV16 E6 protein was robustly expressed in all FCDIIB specimens in BCs, but not in regions without BCs or in control tissue. HPV16 E6 DNA and mRNA were detected in 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 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 new, 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,
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 transmis-

sion 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

References


Instructions
The purpose of this form is to provide readers of your manuscript with information about your other interests that could influence how they receive and understand your work. Each author should submit a separate form and is responsible for the accuracy and completeness of the submitted information. The form is in four parts.

1. **Identifying information.**
   Enter your full name. If you are NOT the main contributing author, please check the box “no” and enter the name of the main contributing author in the space that appears. Provide the requested manuscript information.

2. **The work under consideration for publication.**
   This section asks for information about the work that you have submitted for publication. The time frame for this reporting is that of the work itself, from the initial conception and planning to the present. The requested information is about resources that you received, either directly or indirectly (via your institution), to enable you to complete the work. Checking “No” means that you did the work without receiving any financial support from any third party – that is, the work was supported by funds from the same institution that pays your salary and that institution did not receive third-party funds with which to pay you. If you or your institution received funds from a third party to support the work, such as a government granting agency, charitable foundation or commercial sponsor, check “Yes”.
   Then complete the appropriate boxes to indicate the type of support and whether the payment went to you, or to your institution, or both.

3. **Relevant financial activities outside the submitted work.**
   This section asks about your financial relationships with entities in the bio-medical arena that could be perceived to influence, or that give the appearance of potentially influencing, what you wrote in the submitted work. For example, if your article is about testing an epidermal growth factor receptor (DGFR) antagonist in lung cancer, you should report all associations with entities pursuing diagnostic or therapeutic strategies in cancer in general, not just in the area of EGFR or lung cancer.

   Report all sources of revenue paid (or promised to be paid) directly to you or your institution on your behalf over the 36 months prior to submission of the work. This should include all monies from sources with relevance to the submitted work, not just monies from the entity that sponsored the research. Please note that your interactions with the work’s sponsor that are outside the submitted work should also be listed here. If there is any question, it is usually better to disclose a relationship than not to do so.

   For grants you have received for work outside the submitted work, you should disclose support ONLY from entities that could be perceived to be affected financially by the published work, such as drug companies, or foundations supported by entities that could be perceived to have a financial stake in the outcome. Public funding sources, such as government agencies, charitable foundations or academic institutions, need not be disclosed. For example, if a government agency sponsored a study in which you have been involved and drugs were provided by a pharmaceutical company, you need only list the pharmaceutical company.

4. **Other relationships**
   Use this section to report other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work.
Section #1 Identifying Information

1. Today’s Date: 1/2/2013

2. First Name  Michael     Last Name Wong  Degree MD, PhD

3. Are you the Main Assigned Author?  ☒ Yes     ☐ No

   If no, enter your name as co-author:

4. Manuscript/Article Title: Is Focal Cortical Dysplasia an Infectious Disease?

5. Journal Issue you are submitting for: 13.5

Section #2 The Work Under Consideration for Publication

Did you or your institution at any time receive payment or services from a third party for any aspect of the submitted work (including but not limited to grants, data monitoring board, study design, manuscript preparation, statistical analysis, etc.)?

Complete each row by checking “No” or providing the requested information. If you have more than one relationship just add rows to this table.

<table>
<thead>
<tr>
<th>Type</th>
<th>No</th>
<th>Money Paid to You</th>
<th>Money to Your Institution*</th>
<th>Name of Entity</th>
<th>Comments**</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Grant</td>
<td>☒</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Consulting fee or honorarium</td>
<td>☒</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Support for travel to meetings for the study or other purposes</td>
<td>☒</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Fees for participating in review activities such as data monitoring boards, statistical analysis, end point committees, and the like</td>
<td>☒</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Payment for writing or reviewing the manuscript</td>
<td>☒</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Provision of writing assistance, medicines, equipment, or administrative support.</td>
<td>☒</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Other</td>
<td>☒</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*  This means money that your institution received for your efforts on this study.

**  Use this section to provide any needed explanation.
Section #3  Relevant financial activities outside the submitted work.
Place a check in the appropriate boxes in the table to indicate whether you have financial relationships (regardless of amount of compensation) with entities as described in the instructions. Use one line for each entity; add as many lines as you need by clicking the “Add” box. You should report relationships that were present during the 36 months prior to submission.

Complete each row by checking “No” or providing the requested information. If you have more than one relationship just add rows to this table.

<table>
<thead>
<tr>
<th>Type of relationship (in alphabetical order)</th>
<th>No</th>
<th>Money Paid to You</th>
<th>Money to Your Institution*</th>
<th>Name of Entity</th>
<th>Comments**</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Board membership</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Consultancy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Employment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Expert testimony</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Grants/grants pending</td>
<td></td>
<td>National Institutes of Health</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Department of Defense</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Payment for lectures including service on speakers bureaus</td>
<td></td>
<td></td>
<td></td>
<td>National Institutes of Health</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Department of Defense</td>
<td></td>
</tr>
<tr>
<td>7. Payment for manuscript preparation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. Patents (planned, pending or issued)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9. Royalties</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10. Payment for development of educational presentations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11. Stock/stock options</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12. Travel/accommodations/meeting expenses unrelated to activities listed.**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13. Other (err on the side of full disclosure)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* This means money that your institution received for your efforts.
** For example, if you report a consultancy above there is no need to report travel related to that consultancy on this line.

Section #4 Other relationships
Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?

☒ No other relationships/conditions/circumstances that present a potential conflict of interest.
☐ Yes, the following relationships/conditions/circumstances are present:
Thank you for your assistance.

Epilepsy Currents Editorial Board