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ErbB4 Localization to Interneurons: Clearer Insights into Schizophrenia Pathology

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Schizophrenia is one of the most heritable psychiatric disorders, and haplotype screens of affected families have uncovered several genes linked to this disorder, including the gene encoding neuregulin-1 (*NRG1*) (1). *NRG1* and the receptor tyrosine kinase, ErbB4, through which it exerts its actions, are critical for nervous system development. ErbB4 initiates downstream signaling events that control neuronal progenitor proliferation, interneuron migration from the ganglionic eminences, radial migration of glutamatergic neurons, oligodendrocyte development, and myelination as well as synaptic plasticity in the adult (2). Interestingly, many of these same processes are disrupted in schizophrenia.

Postmortem brains from patients affected by schizophrenia often show a decrease in dendritic spine density on cortical pyramidal neurons relative to control subjects. A number of discrete possible mechanisms may underlie this spine loss. Indeed, the molecular and cellular pathology of schizophrenia involves the misregulation of both glutamatergic and dopaminergic neurotransmitter release and *N*-methyl-D-aspartate (NMDA) receptor hypofunction, all of which could ultimately contribute to the loss of synaptic connections during adolescence.

Locating the site of ErbB4 action in the frontal cortex is crucial to understanding how ErbB4 modulates cortical circuitry on a molecular level. Several studies indicate that the loss of ErbB4 in rodent models leads to reductions in synapse maturation and dendritic spine density (3,4). A major unresolved issue has been whether ErbB4 acts cell autonomously within excitatory postsynaptic spines, presynaptically, or through a more complex circuit of interneurons to affect spine number.

Evidence supporting ErbB4 action at each of these sites has been presented in the literature. For example, some studies have suggested that ErbB4 is upregulated in postsynaptic densities upon *NRG1* stimulation and that it can enhance 2-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) currents and drive dendritic spine maturation (4,5). Other studies have demonstrated that ErbB4 localizes presynaptically to interneuron terminals to promote the formation of inhibitory synapses onto excitatory pyramidal neurons (6,7). Resolving precisely when and where ErbB4 acts to prevent spine loss is critical for understanding the mechanism by which altered ErbB4 function contributes to schizophrenia pathology.

One major difficulty in verifying the site of ErbB4 action has been conflicting data regarding its localization. Previous studies using *in situ* hybridization and immunohistologic

techniques have reported ErbB4 expression in pyramidal cells in the frontal cortex of primates (8,9) as well as presynaptic expression in interneurons (7). However, antibody studies suffer from limitations; antibodies can both fail to interact with their antigen in fixed tissues and cross-react with noncognate antigens. Recognizing these difficulties, the Buonanno laboratory developed several high-specificity antibodies to assess ErbB4 localization in the frontal cortex (10). In work described here, Neddens *et al.* (10) used double-labeling techniques with pyramidal cell- and interneuron-specific markers to show that ErbB4 expression is restricted to interneurons in young adult rodents (aged between 2–4 months), male rhesus monkeys (aged 7 and 12 years), and adult human males (aged 38 and 42 years). Immunoreactivity within neurons was detected in the medial prefrontal cortex of rodents and in the dorsolateral prefrontal cortex in primates. These findings were backed up by demonstrations that ErbB4 messenger RNA was selectively detected in fast-spiking parvalbumin-positive interneurons by single-cell polymerase chain reaction (PCR) but not detected in excitatory pyramidal neurons.

After establishing that interneurons were exclusively labeled in the frontal cortex, Neddens *et al.* (10) further explored which interneuron subpopulations coexpress ErbB4 in primate tissue. They show that ErbB4 is coexpressed primarily (more than 98%) with parvalbumin-positive interneurons, which are largely characterized as chandelier or basket-type interneurons. In contrast, less than 30% of calbindin-positive interneurons, which innervate the dendritic region of principal cells, express ErbB4.

In addition to this finding, ErbB4 localization was found to be primarily postsynaptic, in which excitatory, glutamatergic synapses form onto inhibitory interneurons. Likewise, ErbB4 signal was not detected in the presynaptic compartment in any neurons in the frontal cortex.

Together these findings strongly suggest that dendritic spine loss observed in pyramidal neurons is caused neither by ErbB4 acting within excitatory neurons nor through presynaptic inputs from interneurons. Rather, spine density may be changing as a compensatory mechanism in response to excitatory circuit modulation of interneurons by pyramidal cells. Consistent with this possibility, disruption of glutamatergic inputs from pyramidal cells onto parvalbumin-positive interneurons leads to schizophrenia-like endophenotypes in mouse models (11).

The report by Neddens *et al.* (10) also indicates that the predominant ErbB4 localization to interneurons is conserved across species, including humans. Others have reported ErbB4 expression in pyramidal cells of the nonhuman and human primate frontal cortex, but evidence presented in the report by Neddens *et al.* (10) makes a strong case that, as in the hippocampus, ErbB4 is restricted particularly to parvalbumin-positive interneurons in the prefrontal cortex of rhesus monkey and human brain samples.

Double-labeling techniques exclude the possibility that ErbB4 colocalizes significantly with pyramidal neurons because ErbB4 immunoreactivity does not overlap with pyramidal cell markers. However, in human tissue, staining for inhibitory markers failed, and therefore, identification of labeled cells as interneurons was based on morphological properties. Neddens *et al.* (10) then expanded their analysis to include the macaque, alongside mouse and rat frontal cortex, to show that colocalization with ErbB4 in primates was highest in the parvalbumin-positive subpopulation of interneurons, followed by calretinin-positive and cholecystokinin-positive interneurons.

It is important to point out that the use of mouse models of schizophrenia has suffered from uncertainty regarding whether they adequately model phenotypes seen in human populations. However, the findings reported here that ErbB4 localization is conserved across

brain regions and species should facilitate acceptance of rodents as a relevant model to study the mechanisms by which alterations in ErbB4 function contribute to schizophrenia pathology.

The data presented by Neddens *et al.* (10) provide strong evidence that ErbB4 localizes to interneurons in primates, and this is a major advancement in the field. However, these data must be reconciled with previous reports suggesting that ErbB4 can also function in principal neurons to promote dendritic spine maturation (4,5). It is possible that ErbB4 is expressed in excitatory neurons early in development in which it promotes spine formation and that it is also expressed later in interneurons in which it may affect spine stability through more circuit-wide effects.

In support of this, knockdown of ErbB4 activity in early postnatal (postnatal days 12–16) rat hippocampal pyramidal neurons can decrease AMPA and NMDA synaptic transmission, consequently reducing spine density and size (4). Here it is important to point out that Neddens *et al.* (10) did not detect ErbB4 expression in excitatory neurons in single-cell PCR assays from rodents of this age. Could it be possible that ErbB4 is expressed in these cells at sub-threshold levels for PCR detection?

The ultimate resolution of this important issue will require selective inactivation of ErbB4 function in distinct neuronal populations *in vivo* and assessment of the effects on spine formation and stability. This approach should pinpoint where ErbB4 is acting to control spine stability. Indeed, selective ablation of ErbB4 from parvalbumin-positive interneurons has been shown to reduce the strength and number of glutamatergic synapses onto interneurons (12). Fully understanding the site of ErbB4 action will greatly further our understanding of how it regulates dendritic spine formation and stability. In doing so, new ways can be found to identify those at risk for schizophrenia and possibly prevent the onset of pathology. The work of Neddens *et al.* (10) is an important step in this direction.

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