

VEGF regulate Epo production by liver cells²? As these cells do not express VEGF receptors, VEGF seems to indirectly regulate Epo production through release of a—yet unknown—suppressor of Epo production. Only minimal VEGF levels were required to affect Epo production, suggesting a sensitive physiological regulatory pathway. Surprisingly, however, VEGF suppressed the production of Epo, although most previous data indicated that VEGF and Epo amplified each other's activity¹⁶. The discoveries of Tam *et al.* open new avenues to explore this exciting regulation further in the future.

Curiously, Tam *et al.* found that gene transfer of Flk1-Fc (a VEGF-selective trap) only induced half of the erythropoietic response caused by Flt1-Fc (a trap of VEGF, VEGF-B and PlGF). These observations raise the question of whether other VEGF family members besides VEGF are

involved in hepatic Epo synthesis. PlGF, for one, does not seem to be, according to Tam *et al.*². Thus, the use of antibodies against PlGF as an antiangiogenic therapy for cancer may be a suitable alternative or adjunct to VEGF blockade—as PlGF blockade might not affect Epo expression, and could thereby possibly reduce the risk of resistance to antiangiogenic therapy.

In the foreseeable future, there will likely be an exponential rise in the use of antiangiogenic agents in the clinic for a variety of illnesses. The innovative work of Tam *et al.* highlights the value of continuously exploring the molecular mechanisms underlying current interventional approaches, as these will facilitate the strategic design of safer, more effective and durable therapies. For the near future, measuring Epo levels in cancer patients treated with antiangiogenic agents should be insightful.

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Schizophrenia: signals from the other side

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Animal models of schizophrenia are incomplete, and human brain tissue has been difficult to study. Results from postmortem human tissue begin to overcome these hurdles and find a role for modulation of NMDA type glutamate receptors (pages 824–828).

Schizophrenia is a complex developmental disorder that affects many regions of the cerebral cortex and several nuclei deep in the brain. One explanation for the underlying pathophysiology of schizophrenia, based on psychopharmacology, holds that there is an imbalance between dopaminergic and glutamatergic synaptic transmission. Too much dopamine in critical regions of the brain, or too little glutamate, can precipitate behaviors that mimic the positive and negative symptoms of schizophrenia as well as the cognitive deficits. Dopamine antagonists are among the best available antischizophrenia drugs.

Recent attention has focused on the glutamate side of the equation. But glutamate is the major excitatory transmitter in the brain, and global hypotheses regarding glutamate function are not helpful in thinking about molecular or circuit explanations. Attention has turned to modulators of glutamate

function that may act more selectively at specific synapses and at specific times during development and in the adult brain. Modulators have been identified that alter the amount of glutamate released at synapses, that affect reuptake of glutamate and that modulate glutamate receptor function¹.

Products of the NRG1 gene (there are four) have been shown to regulate the type of glutamate receptor that is activated by NMDA in cell cultures and slices prepared from rat prefrontal cortex². In this issue, Hahn *et al.*³ have used postmortem human tissue to show that NRG1 reduces the tyrosine phosphorylation of NMDA receptors (NMDARs), a modification that is triggered by the binding of NMDA or glutamate.

It is remarkable that the authors were able to study delicate enzyme reactions in postmortem tissue, as one must control for shifts in pH, energy substrates and ion composition. This is a welcome advance in the absence of convincing animal models for complex disorders of human thought and social cognition, such as schizophrenia and autism.

The effect of NRG on NMDA glutamate receptors is associated with an increase in phosphorylation of erbB4, the principal NRG1 receptor in the prefrontal cortex. Hahn *et al.* also found an enhanced association between erbB4 and PSD95, a component of the postsynaptic apparatus. The binding of PSD95 is probably involved in the enhanced activation of erbB4 (refs. 4,5), and it may also provide a physical link between erbB4 and the NMDAR (Fig. 1).

Even in the absence of added NRG, the levels of phosphorylation of activated NMDARs were reduced in brains from schizophrenic subjects, and Hahn *et al.* suggest that enhanced endogenous NRG1-erbB4 signaling may be responsible for NMDAR hypofunction in the disease state. The effect of added NRG was, in fact, greater in tissue from individuals with schizophrenia than in controls.

The new findings dovetail with previous work suggesting a role for NRG1 in schizophrenia. In this work, a linkage study of an Icelandic population that identified a 'risk haplotype' in the 5' end of the 1.2 Mb NRG1

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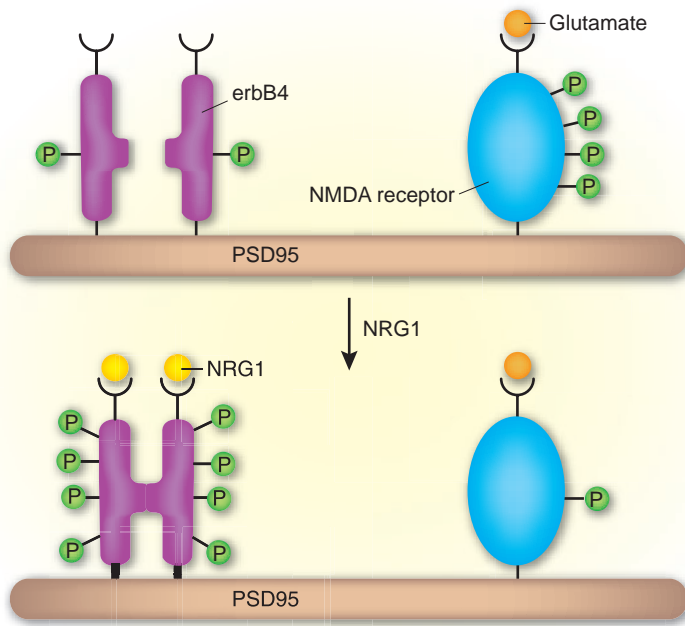


Figure 1 A proposed mechanism for NRG1 induced hypophosphorylation of glutamate-activated NMDA type glutamate receptors in schizophrenia; this mechanism is based on studies of postmortem human prefrontal cortex by Hahn *et al.*³ On binding of NRG1, erbB4 is hyperphosphorylated (P), presumably because the interaction between PSD95 and erbB4 is enhanced in schizophrenia. This hyperphosphorylation of erbB4 leads to a hypophosphorylation of activated NMDA type receptors. Both erbB4 and NMDA type receptors bind to PSD95.

gene⁶. The NRG1 gene is complex, with as many as nine different promoters and at least fourteen different predicted protein isoforms⁷. The five single-nucleotide polymorphisms (SNPs) and two microsatellites within the haplotype are located upstream of most, if not all, of the coding exons of known NRG1 isoforms, and to date, no coding mutations have been reported.

It has been suggested, therefore, that a change in the expression of NRG1 rather than a specific mutation in a coding region may contribute to the risk for schizophrenia (although sequence data suggests that novel isoforms of the NRG1 gene may exist)⁷.

Several questions are raised by these provocative findings. First, what changes in NRG1 or erbB4 or both might lead to increased phosphorylation of erbB4 and to enhanced interaction with PSD95—and ultimately, to suppression of NMDAR function? The hyperphosphorylation of erbB4 and the hypophosphorylation of NMDAR were evident, even though western blots indicated that levels of NRG1, erbB4 and PSD95 were not increased. Other studies, however, indicate an increase in NRG1 (type 1) mRNA in human prefrontal cortex from schizophrenia subjects compared to age-matched controls⁸ and in human lymphocytes⁹.

More precise measures of NRG1 isoforms, of erbB4 and of other erbBs that may dimerize with erbB4 are needed. The NRG1 EGF-like domain that is common to all members of the NRG1 family is sufficient to activate erbB receptor kinases, but the different NRG1 isoforms may have different distributions within cells and in the extracellular matrix.

Second, at the level of the NMDA receptor, it is not clear whether tyrosine phosphorylation results in an increase or a decrease in current flow through the NMDA ion channel. Although NRG decreases NMDA currents acutely, long-term application of NRG can increase the expression of at least one NMDAR subunit (2C)¹⁰. NRG1 effects on nicotinic acetylcholine receptors composed of alpha 7 subunits present a similar picture. Application of NRG1 to cultured hippocampal neurons for 1–2 days enhances alpha 7 currents¹¹, but acute application suppresses the same currents (Q. Chang & G.D.F., unpublished data). The distinction between acute and chronic exposure will depend on a better knowledge of the kinetics of NRG release from endogenous stores.

We must also learn more about the cast of characters in the various signaling cascades triggered by NRG. The complexity of NRG signaling is hinted at by the growing number

of reports of the effects of NRG on neuronal and glial precursors—including proliferation, cell fate determination, maturation and migration. NRG also has multiple effects on ligand and voltage-gated ion channels and—ultimately—on synaptic efficacy^{12,13}.

Moreover, it is not yet clear whether modulation of NMDARs leads to increased or decreased output of key neural circuits. More studies of functional anatomy at the level of simple circuits with millisecond resolution are needed. This is a difficult but essential step if we are to understand the implications of glutamate receptor phosphorylation for even the simplest altered behaviors of schizophrenia.

Perhaps most importantly, we must recognize the complexity of schizophrenia. It may be more productive to concentrate on particular aspects of the broad phenotype. Categorizing behavioral and biochemical endophenotypes that, in the aggregate, define what we mean by ‘schizophrenia’ may aid in identifying susceptibility genes, and they may aid attempts to analyze the functional anatomy of the disordered nervous system. One must search for the earliest signs of the disorder to dissociate the initial insults from later, reactive, perhaps compensatory changes brought about by the remarkable plasticity of the human brain.

Next steps in our understanding of molecular and cellular aspects of schizophrenia must come to grips with the complexity at each level of analysis. Insights at one level may shed light on approaches at other levels. In this spirit, the Hahn *et al.* study, based on molecular analyses of human postmortem prefrontal cortex tissue, is a welcome addition.

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