Neurofibromatosis type 1 (NF1) is a common autosomal dominant neurogenetic disorder in which affected patients develop both benign and malignant tumors at an increased frequency. Neurofibromatosis type 1 affects approximately 1 in 3000 individuals worldwide, without regard to sex, race, or ethnic background. The hallmark of the clinical disorder is the development of pigmentary lesions (cafe au lait spots, skinfold freckling, and Lisch nodules), distinctive skeletal lesions (sphenoid wing dysplasia and pseudoarthrosis), and tumors, such as optic pathway gliomas and neurofibromas. In addition to these clinical features, 40% to 60% of children with NF1 manifest specific learning disabilities, including attention deficit hyperactivity disorder and deficits in visuospatial processing.

The identification of disease genes through powerful genetic approaches, such as positional cloning, holds the promise for a better understanding of the pathogenesis of neurogenetic diseases. The NF1 gene was identified by positional cloning in 1990, and its protein product, termed neurofibromin, was isolated in 1991. Neurofibromin is expressed in neurons, Schwann cells, the adrenal medulla, and white blood cells. It has been associated with microtubules in some cell types and functions in other cell types as a negative regulator of the proto-oncogene p21-ras, which is a critical molecule in many intracellular signaling pathways and transduces both growth-promoting and growth-arrest signals.

With the gene in hand and the protein identified, it then became possible to begin to address the molecular pathogenesis of NF1 and develop therapies specifically targeted for patients with this disorder. In 1995, the National Neurofibromatosis Foundation and Cold Spring Harbor Laboratories sponsored a symposium to challenge clinicians and scientists alike to define the areas of clinical and basic science research important for improving our understanding of NF1 disease pathogenesis and treatment. In particular, the charge was to begin to translate what has been learned from the laboratory and in the clinic into more effective management strategies for individuals affected with NF1. The 4 major areas identified by this group were (1) learning disabilities, (2) optic pathway gliomas, (3) neurofibromas, and (4) other malignancies. In this review, I focus on the progress and future directions of NF1 clinical and basic science research in the context of the Banbury Conference initiative.

LEARNING DISABILITIES

Forty to sixty percent of children with NF1 have some form of cognitive deficit or learning disability that interferes with school performance. The frequency of learning disabilities in the context of NF1 suggests a role for the NF1 gene in learning and memory. Children with NF1 often have attention deficit hyperactivity disorder, as well as difficulties with visuospatial learning. In addition, magnetic resonance imaging scans of the brain of some children with NF1 demonstrate high signal intensity lesions on T2-weighted images. These unidentified bright objects (UBOs) are sometimes interpreted as hamartomas; however, pathologic proof for this notion is lacking. Unidentified bright ob-
projects most likely represent areas of increased water content with surrounding gliosis. Some groups have found a correlation between the presence of UBOs and a left shift in the full-scale IQ in that children whose magnetic resonance imaging scans showed evidence of UBOs had mean IQ scores around 85, compared with their UBO-negative counterparts with mean IQs around 100. These findings have not been replicated in other studies; however, in one report, a specific correlation was demonstrated between the presence of UBOs in the thalamus and learning disabilities in children with NF1.

Given the high incidence of learning disabilities in children with NF1, what do we know about the role of the NF1 gene in brain development and neuronal function? Neurofibromin has been shown to be expressed in all neurons in the central nervous system, where it is localized to both dendritic processes and axons. In the brain, neurofibromin has been shown to associate with cytoplasmic microtubules, raising the possibility that neurofibromin might be involved in signaling pathways within neurons. In addition, we and others have described the existence of a neuronal-specific NF1 RNA isoform containing exon 9a, generated by alternative messenger RNA splicing. NF1 messenger RNA containing exon 9a is restricted to neurons of the forebrain, hippocampus, thalamus, hypothalamus, and striatum, with no expression in the brainstem, spinal cord, or cerebellum. Furthermore, the expression of exon 9a-containing NF1 messenger RNA is developmentally regulated, with expression first detectable in early postnatal life. These findings argue that a specific form of neurofibromin might be important in the development of the nervous system.

Other insights into the role of the NF1 gene in nervous system function and learning and memory have come from studies on genetically engineered mice and drosophila. The results from studies on mice genetically engineered to lack NF1 gene expression through targeted disruption of one or both copies of their NF1 genes have provided some fascinating clues. First, neurons cultured from mice defective in NF1 gene expression no longer require neurotrophins for survival in vitro. These results suggest that the loss of neurofibromin expression in neurons eliminates the need for survival factors otherwise required for their maintenance. Alternatively, it is possible that the loss of neurofibromin expression results in developmental delay of neurons, and that this delay results in a less differentiated cell that has not yet developed a requirement for neurotrophin support. If this latter hypothesis were true, it would predict that neurons in the brains of individuals with NF1 may be developmentally immature, so that the formation of proper connections at the appropriate times during the development of the central nervous system is delayed. This delayed neuronal development could result in the improper wiring of the central nervous system and contribute to the development of cognitive deficits.

In studies on mice heterozygous for an NF1 gene mutation (NF1+/−) in which 50% of the wild-type levels of neurofibromin are expressed, defects in spatial learning have been demonstrated. In these studies, NF1+/− mice do not perform as well as their wild-type littermates in water maze tests of spatial learning, but retain their capacity to learn through other mechanisms (eg, fear conditioning). Since the neurons in brains from NF1+/- mice genetically resemble the neurons from patients with NF1, the finding of similar learning disabilities suggests that reduction of neurofibromin expression in the brain is sufficient for the development of learning disabilities. The contribution of reduced neurofibromin expression in astrocytes relevant to normal brain development has not yet been addressed.

Using the fruit fly as another model for studying the contribution of the NF1 gene to nervous system development and function, drosophila mutants were created that lack NF1 gene expression. These flies are smaller than their wild-type counterparts and demonstrate subtle behavioral abnormalities. Moreover, these NF1 mutant drosophila have deficits similar to other drosophila mutants with defects in the protein kinase A signaling pathway. Analysis of these NF1 mutants demonstrated abnormalities in peptide hormone stimulation–induced neuromuscular junction potassium channel currents. These results collectively suggest that neurofibromin in flies might be important for regulating neuronal activity through a unique pathway involving protein kinase A. Should the protein kinase A pathway prove relevant in neurofibromin neuronal signaling in mammals, this finding may have implications for future therapies directed at the learning disabilities of children with NF1.

OPTIC PATHWAY GLIOMAS

Fifteen percent of children with NF1 harbor a benign, astrocytic tumor involving the optic pathway. These tumors represent pilocytic astrocytomas that rarely progress to malignant tumors. Optic pathway tumors in children with NF1 are most commonly seen in children younger than 6 years of age. Half of the children with optic pathway tumors are asymptomatic at the time of diagnosis, yet few will go on to develop further sequelae or visual loss. The clear challenge for clinicians and scientists alike with regard to optic pathway tumors is to determine which tumors in children with NF1 will remain dormant, and which are likely to progress and require aggressive treatment. Guidelines for the evaluation and management of children with optic pathway tumors have recently been formulated.

The role of the NF1 gene as a negative growth regulator for astrocytes has been studied over the past few years. It has been demonstrated that neurofibromin expression is low in resting astrocytes but can be dramatically up-regulated in astrocytes in response to activation of the protein kinase A pathway, cytokine stimulation, and cerebral ischemia. Analysis of a limited series of brains from individuals with NF1 has demonstrated an increased number of glial fibrillary acidic protein immunoreactive astrocytes in the brain. These results have been confirmed in NF1+/- mouse brains. The fact that neurofibromin expression can be dramatically modulated by several stimuli both in vitro and in vivo raises the possibility that neurofibromin has a specific role in astrocytes in response to certain physiological stimuli. In this regard, loss of neurofibromin expression in astrocytes might only result in tumor formation in the presence of...
certain physiological stimuli (eg, cerebral ischemia or brain injury). Future studies aimed at defining the role of neurofibromin as a negative growth regulator for astrocytes may provide the necessary insights required to begin to develop targeted therapies for these tumors in children with NF1.

NEUROFIBROMAS

Nearly all individuals affected with NF1 will develop neurofibromas at some time during adulthood. Although these neurofibromas do not progress to malignant neoplasms, a subset of neurofibromas, termed plexiform neurofibromas, carry a 5% lifetime risk of malignant transformation. The plexiform neurofibroma is thought to represent a congenital lesion composed predominantly of Schwann cells and fibroblasts. Because of the serpiginous nature of these tumors and their significant vascularity, complete surgical resection is often not possible. As is true with the optic pathway tumors, identifying those individuals with NF1 and plexiform neurofibromas likely to progress to malignant peripheral nerve sheath tumors represents the challenge for clinicians and scientists.

Neurofibromin is highly expressed in Schwann cells. A recent study has demonstrated that loss of NF1 gene expression occurs in the benign neurofibroma. At this time, it is not clear whether the development of neurofibromas results from the loss of neurofibromin expression in Schwann cells or fibroblasts, although it is likely that the responsible cell type is the Schwann cell. In studies on Schwann cells and fibroblasts from mice with a targeted disruption of the NF1 gene, defects in cellular functioning of both fibroblasts and Schwann cells have been demonstrated. In particular, Schwann cells derived from mice lacking NF1 expression have increased levels of ras activation consistent with the role of neurofibromin as a negative ras regulator. In addition, these Schwann cells are angiogenic and have an increased growth rate compared with normal Schwann cells. These results collectively suggest that alterations in NF1 gene expression have profound effects on the biological function of the Schwann cell. However, future studies will be required to determine how loss of neurofibromin expression leads to the formation of neurofibromas.

CONCLUSIONS

It has been an exciting several years for NF1, both in terms of clinical research and basic bench science. Several key areas for future investigation have been identified, and a framework for the diagnosis and treatment of patients with NF1 has been devised. It is anticipated that the next several years will witness the development and testing of targeted therapies based on our improved understanding of the clinical behavior and the molecular biological features of neurofibromin in neurons, astrocytes, and Schwann cells.

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REFERENCES