International Consortium Summary (2000)

NNFF International Consortium for the Molecular Biology of NF1 and NF2 Meets in Aspen, Co.

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The 2000 international meeting of the neurofibromatosis research community was held in Aspen, Colorado from June 4 -7, 2000. This meeting continues a tradition that was established in 1986 when researchers brought together by the NNFF formed "The International Consortium For The Molecular Biology of NF1 and NF2" to identify genes that are mutated in individuals with NF1 and NF2. These efforts facilitated the identification of the NF1 gene in 1990, and the NF2 gene in 1993. Since these genes were discovered, participants in Consortium meetings have reported the most recent research in the areas of natural history, mutation detection, functional studies of the proteins produced by the NF1 and NF2 genes, the development of animal models, and new treatment strategies. By bringing a diverse group of investigators together to discuss ideas and form collaborations, Consortium meetings continue to facilitate research into the most important clinical and scientific questions in NF research.

Another important function of Consortium meetings is to bring distinguished researchers from outside the immediate NF field whose insights and discoveries have potential impact for NF. This year's meeting was highly successful, boasting the largest numbers of submitted abstracts (68) and attendees (146). Prominent outside participants at this year's meeting included Drs. Ronald DePinho (Harvard/Dana Farber Cancer Institute), Jeffrey Settleman (Harvard/ Massachusetts General Hospital), David Stokoe (University of California, San Francisco) and scientists from several pharmaceutical companies.

A new and highly successful innovation at this year’s meeting involved awards from the NNFF to 10 young investigators from around the world to underwrite the costs associated with attending the meeting.This allowed these individuals to interact with leading NF researchers, and to present their ideas at the meeting.

Keynote Address
The meeting opened with a lecture enti-tled “NF at the Millennium” in which Dr. James Gusella (Harvard/Massachusetts General Hospital) reviewed the enormous progress made in understanding NF1 and NF2 over the past 2 decades, and articu-lated several pressing questions that remain. Dr. Gusella is a distinguished, long-term member of the NF research community whose pioneering studies helped lay the groundwork for the cloning of the NF1 gene and who led one of the two research teams that identified the NF2 gene (Dr. Guy Rouleau, McGill Uni-versity, led the other group). Dr. Gusella reminded participants that as the field develops in response to the identification of the NF1 and NF2 genes, it is essential that researchers continue to learn from patients with NF by integrating clinical observations with studies of gene mutations and gene function. Describing what happens to patients with NF contributes specific insights, while experi-ments performed in the laboratory provide a different type of information. These approaches must ultimately fuse to achieve a complete understanding of NF1 and NF2.

Experimental Therapeutics Session
In contrast to previous years, the opening session of the meeting was devoted to therapeutics - a topic that has traditional-ly been left to the final discussion of ‘future goals’. This scheduling decision reflects the commitment of NF researchers to develop new treatments that target the underlying biochemical abnormalities associ-ated with NF gene mutations. Because more is known about how neurofibromin, the protein produced by the NF1 gene, functions in cells, this

process is more advanced for NF1. When the NF1 gene was cloned, it was found to closely resemble other genes in yeast, flies, and humans.The yeast genes were known to “turn off ” the activity of a pro-tein called Ras. Ras plays an important role in many cells by relaying signals that influence growth from the surface to the nucleus. Because mutations that result in overactive Ras proteins also contribute to many types of cancer, researchers have been working intensively to develop new treatments that can interfere with the function of Ras. Such treatments may also benefit patients with NF1. Dr. Jackson Gibbs (Merck Cancer Research Laboratory) organized the therapeutics session. Presentations included a discus-sion of the use of inhibitors of an enzyme called farnesyltransferase, which modifies Ras and many other proteins to increase their activity in cells. Several far-nesyltransferase inhibitors (FTIs) are being studied in cancer patients. Dr. Wanda Selzer (Wilford Hall USAF Med-ical Center and National Cancer Institute) described the results of a pre-liminary trial of an FTI developed by Janssen on pediatric NF1 patients with plexiform neurofibromas. Although none of the tumors decreased significantly in size, some patients felt better and there were few side effects. A follow-up study is planned.Three other speakers described other drugs including (1) an inhibitor of a protein called MEK that transmits growth-promoting signals from Ras to the nucleus (Dr. Judy Leopold, Parke-Davis); (2) another inhibitor of the epidermal growth factor receptor (EGFR), which may stimulate abnormal growth in certain cells (Dr. James Moyer, Pfizer); and, (3) compounds that block new blood vessel formation in tumors (Dr. Gerald McMahon, Sugen). All of these agents are in the early testing phase in patients with advanced cancer. If these results are promising, they may prove beneficial in patients with NF1 based on scientific results described below.

NF1 Research

NF1 researchers have long tried to under-stand how mutations occur in the NF1 gene. Two researchers, Dr. Lopez-Correa, Leuven, Belgium and Dr. Dorscher, Univer-sity of Washington, independently discov-ered that sequences surrounding the NF1 gene called NF1-REPs may promote exchanges between different chromosomes and thus increase the probability of new gene mutations.The large size and complex structure of the NF1 gene necessitates strategies capable of detecting many different mutations. Promising data describing the efficacy of a combined approach that utilizes protein transcription-translation (PTT) together with other methods were presented (Dr. Ludwine Messaiaen, University of Ghent, Belgium).This combined approach led to the detection of mutations in 95% of the NF1 patients.

Two other important themes in NF1 research involve trying to understand (1) how abnormal responses to growth factors contribute to the problems that occur in individuals with NF1, and (2) if cells that have one mutated and one nor-mal copy of the NF1 gene show subtle defects in growth control. Several labs have discovered enhanced sensitivity to growth factors of specific cells that have mutations in one or in both copies of the NF1 gene. Keynote speaker Dr. David Stokoe (University of California, San Fran-cisco) provided an overview of signaling pathways involved in cell survival and pro-liferation. It is known from the work of Drs. Vogel (Louisiana State University) and Parada (University of Texas SW) that immature neurons from NF1 mutant mice exhibit increased survival compared to normal neurons. Recent studies presented at this meeting indicate that fibroblasts and brain astrocytes (Dr. David Gutmann, Washington University, St. Louis), mast cells (Ingram), and immature bone marrow cells (Dr. David Largaespada, Uni-versity of Minnesota) from NF1 mutant mice are sensitive to otherwise limiting levels of growth factors. Two presentations from studies performed in cultured mouse cells and human tumors suggest that over-activation of the EGF receptor may stimulate the growth of malignant peripheral nerve sheath (MPNST) tumors (Drs. Jeffrey DeClue, National Cancer Institute and Larry Sherman, University of Cincinnati).

Another question facing NF1 researchers is whether neurofibromin solely controls cell growth through its effect on the Ras protein. In support of a Ras-dependent mechanism, introducing a small piece of neurofibromin that interacts with Ras into either cultured mouse fibroblasts or bone marrow cells that lack the NF1 gene restored normal growth (Hiatt). In contrast, studies presented by Drs. Andre Bernards (Harvard/MGH) and Hui Fu Guo (CSHL) of a gene related to NF1 in fruit flies suggests that neurofibromin may function in a Ras-independent manner in neural cells.

Dr. Tyler Jacks (MIT) and Drs. Camilynn Brannan (University of Florida) and Neal Copeland (NCI) and Luis Parada independently developed lines of mice in the early 1990s that carry a mutation in one copy of the NF1 gene. Studies in these mutant mice have provided many novel insights, some of which were discussed earlier in this review. Mice with one abnormal NF1 gene carry a similar genetic mutation as people with NF1, and they are prone to some of the tumors that occur in NF1 patients. However, these mice do not develop neurofibromas. Another problem is that mouse embryos that carry mutations in both copies of the NF1 gene die before birth, thereby precluding important stud-ies on the effects of inactivating both copies of the gene in specific tissues. In an important advance, Drs. Yuan Zhu (University of Texas SW) and Luis Parada have engineered a mutation into mice that allows researchers to mutate the NF1 gene in specific tissues. Preliminary studies presented at the meeting indicate that NF1 plays an important role in the devel-opment of different parts of the brain, and that inactivating the gene promotes tumor formation.This line of mice should prove extremely valuable for a variety of studies over the next few years.

One curious aspect of NF1 is that some individuals have many severe complications of the disease, while others have relatively few. Studies performed to date do not support a correlation between the type of NF1 gene mutation that a person carries and the problems they experience. Instead, it seems likely that other genes might interact with NF1 to modify the kind of disease that devel-ops. Mouse genetics provide a powerful tool for exploring how genes other than NF1 might affect what happens to patients with NF1.Two approaches have been taken in an effort to discover genes that might interact with NF1 in tumor for-mation. In the first, a virus that causes mutations in immature blood cells was used to find and identify modifiers of leukemia in NF1 mutant mice (Dr. Susan Blaydes, University of Florida). Similarly, an effort to identify genetic loci that modify tumor development in different strains of NF1 mutant mice was presented (Dr. Karlyne Reilly, MIT). Preliminary data suggest that other genes exist that influence the risk of developing astrocytoma, leukemia, and sarcoma in mice. Efforts are underway to map these modifier loci.

NF2 Research

The protein produced by the NF2 gene has been named merlin or schwannomin (hereafter referred to as merlin for simplicity). This protein is a located at the surface of the cell and is related to the ezrin/radixin/moesin (ERM) family of pro-teins. These findings suggest that merlin controls cell growth by regulating chemical reactions that occur within the plasma membrane on the surface of the cell. NF2 researchers have been working intensively to identify the proteins that merlin inter-acts with, and to understand how these interactions regulate growth mechanisms by which this is achieved. Previous studies have suggested that merlin exists in two forms; a "closed" form in which it actively controls other proteins, and an "open" conformation in which merlin is inactive. New data was presented that suggests that merlin can, in fact, bind directly to a protein called actin when it is in the open conformation. Actin plays an important role in controlling the shape and movement of many types of cells. Other studies focused on interactions between merlin and the protein EBP50/NHE-RF, which may link merlin function to pump-ing ions across the cell membrane (Dr. Thorsten Wiederhold, Harvard/MGH). EBP50/NHE-RF, in turn, binds via its PDZ domain to a number of other proteins in the plasma membrane, including the b2-adrenergic receptor and a new molecule dubbed EPI-64/65 that resembles yeast proteins known to be involved in control-ling actin (Dr. David Reczek, Cornell Uni-versity). Links from merlin/ERMs through PDZ-domain containing proteins to mem-brane receptors in therefore emerging as recurring theme. These interactions are likely to be important in the ability of merlin to control cell growth and to pre-vent tumor formation.

Among known membrane-binding partners of merlin/ERMs is the hyaluronic acid receptor, CD44. New evidence was presented that the growth suppressing function of merlin is dependent on CD44 function (Morrison).Turning merlin expression on in malignant schwannoma cells stopped the growth of these cells when they were confluent (that is, when they had covered the bottom of a culture dish). This growth arrest was blocked by treatments that would be predicted to block

merlin:CD44 interaction or CD44 function. Given the observations that mer-lin phosphorylation is regulated by cell confluence and that NF2-deficient cells grow to a higher saturation density than their normal counterparts (Dr. Andrea McClatchey, Harvard/MGH), these studies suggest an interesting link between merlin function and contact inhibition – a prop-erty that is often lost by tumor cells.

In addition, several new proteins that interact with merlin were reported. For example, an interaction between merlin and the focal adhesion protein paxillin in Schwann cells, was reported (Dr. Jerome Ricard, University of Central Florida). This is intriguing given published studies that suggest a role for merlin in cell adhesion. Another intriguing interacting partner, HRS (HGF-regulated protein kinase sub-strate), was identified (Dr. Daniel Scoles, UCLA). HRS is thought to be involved in endocytic sorting of molecules that trans-fer signals to the nucleus and may regulate a class of these proteins called STATs. Switching from the inactive "open" form to the active "closed" conformation appears to play an important role in how merlin regulates cell growth.

Cellular enzymes that add or remove phosphates to proteins control the activity of many proteins, and this seems to apply to merlin as well. As adding phosphate groups to merlin are likely to be important in regulating merlin/ERM activi-ty, the study of what proteins perform this function and how they are regulated is an important priority in NF2 research. Sever-al recent lines of evidence link phosphory-lation and activation of the ERM proteins to signaling by members of the Rho family of small GTPases, which are known to be involved in actin cytoskeleton reorganiza-tion. Keynote speaker Dr. Jeff Settleman (Harvard/ MGH), whose laboratory stud-ies the Rho GTPases in both mammals and fruit flies, gave an overview of Rho GTPase signaling and described his analy-sis of the function of the Rho regulator p190-RhoGAP in the mouse nervous sys-tem. A new functional connection between Rho and ERM functions was then described (Lamb). While previous work indicates that activation of Rho-mediated signaling leads to phosphoryla-tion and 'activation' of the ERM proteins, new studies suggest that the ERM proteins themselves are required for Rho activation by a 'feed-forward' mechanism. In addition, evidence for an interaction between the ERM proteins and hamartin, the product of the tuberous sclerosis type I tumor suppressor locus, was presented. A link between hamartin, and the ERMs appears to be important in regulating cell adhesion and controlling Rho activity.

Merlin phosphorylation is regulated under different conditions, including growth fac-tor availability, cell adhesion and, interest-ingly, cell density (Drs. Shaw, McClatchey, Jacks, 1998). In contrast to the ERMs, mer-lin appears to be regulated by the Rho- family member, Rac (McClatchey). More-over, the properties of NF2-deficient cells suggest that Rac signaling may be elevated in some contexts in the absence of NF2. Rac-induced phosphorylation of merlin would be predicted to 'inactivate' it. Thus, like the Rho-ERM relationship, a 'feed-for-ward' mechanism whereby Rac induces phosphorylation and inactivation of mer-lin, relieving its negative regulation of Rac signaling can be hypothesized.

As for NF1, many laboratories have been working to develop animal models of NF2. Originally NF2 mutant mice were generated, and as for NF1+/- mice, were found to be cancer prone. However, these mice do not develop the Schwann cell tumors that are the hallmark of NF2. Instead they develop cancers of the bone and liver (McClatchey). However, recently, targeted inactivation of both copies of the NF2 gene in Schwann cells produces excessive growth of Schwann cells, and, occasionally, schwannoma tumors (Dr. Marco Giovannini CEPH, Paris, France). The same researchers have succeeded in producing meningiomas in NF2 mice. Thus, the two hallmark tumors of human NF2, schwannomas and meningiomas, can be recapitulated in mice by removing NF2 gene function.

Summary

The NF research community includes cli-nicians, clinical investigators, and basic researchers working at academic institutions and in the pharmaceutical industry. Consortium meetings provide a valuable forum for these groups to share perspectives and to discuss new research data.

Several strong themes have emerged.The first of these is the impor-tance of accurately describing the natural history of NF1 and NF2 so that the impact of new therapies can be ascertained.Two large studies are under-way with support from the US Army NF Research Program. A second theme involves the intense effort being directed toward developing and characterizing bet-ter animal models, which mimic the most important features of human NF1 and NF2.The generation of conditional mutant alleles of both genes in the mouse should greatly facilitate these efforts. Substantial progress has already been made; for example, NF2 mice develop meningiomas and schwannomas, and NF1 mice develop myeloid leukemia, pheochromocytoma, and MPNST. Producing a mouse model of neurofibroma remains a major challenge. Third, extensive progress has been made toward understanding the molecular func-tions of neurofibromin and merlin in cells. These basic studies are absolutely essen-tial for discovering the pathways in cells that will prove amenable to therapeutic intervention. Finally, there is increasing emphasis on developing and testing new treatments for NF1 and NF2. New thera-pies for NF1 are likely to emerge sooner because more is understood about neu-rofibromin function, and because of the intense interest in Ras within the pharma-ceutical industry. Progress in developing new treatments will be greatly enhanced by the existence of excellent mouse mod-els for pilot testing of promising new agents. Indeed, the US Army NF Research Program recently agreed to fund a collab-orative effort in this area. In general, patients with NF and their families can continue to look forward to an enhanced understanding of the underlying mechanisms of NF1 and NF2 and, impor-tantly, to increasing emphasis on devising and testing new therapies based on this knowledge.

NF Glossary

Neurofibromatosis (NF): Genetic disorder that causes tumors to grow on the nerves anywhere in the body. NF can sometimes affect other areas of the body such as bones and skin.

Chromosome: One of the thread-like packages of genes and other DNA present in the nucleus of all cells in living organisms. Gene:The fundamental physical unit of heredity passed from parent to child.

Genes are pieces of DNA— most genes contain the information for making specific proteins in the body.

DNA:This living instructional manual is present in the form of a chemical molecule called deoxyribonucleic acid (DNA).