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Role of the *neurofibromatosis Type 2* gene in the development of tumors of the nervous system

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Germ line and somatic mutations in the *neurofibromatosis Type 2 (NF2)* tumor suppressor gene predispose individuals to tumors of the nervous system, including schwannomas and meningiomas. Since identification of the *NF2* gene more than a decade ago, a large body of information has been collected on the nature and consequences of these alterations in patients with NF2 and in individuals in whom sporadic tumors associated with NF2 develop. The catalog of mutations identified thus far has facilitated extensive genetic analysis, including studies of patients with mosaicism and phenotype–genotype correlations, and has also led to experiments that have begun to unravel the molecular biology of the *NF2* gene and its role in tumorigenesis. The authors describe some of the most significant findings in *NF2* genetics and biology over the last decade.

KEY WORDS • neurofibromatosis Type 2 • genetics • molecular biology

OVERVIEW

The *NF2* tumor suppressor gene, which is located on chromosome 22, was identified in 1993.[55,69] The clinical syndrome NF2 (previously termed central neurofibromatosis or bilateral acoustic neurofibromatosis) had been described more than 170 years earlier.[73] It became clear during the twentieth century (predominantly during the latter half) that NF2 was a distinct clinical entity from NF1 (von Recklinghausen disease or peripheral neurofibromatosis).[10,15,16,24,37,51,72] The *NF2* gene codes for a protein named separately by the two groups who identified it in 1993; that is, schwannomin or merlin. *Schwannomin* was proposed as a name for the *NF2* gene because of its inactivation in schwannomas. *Merlin* was suggested because of the homology of the *NF2* gene to the ERM family of proteins: moesin, ezrin, and radixin. Nevertheless, neither term has been universally accepted, and it would appear that a hybrid name is required. In an attempt to incorporate both names and to avoid further confusion in the literature, we suggest that the gene be referred to as *schmerlin*.

There have been numerous publications in which alterations in the *NF2* gene have been described (both in patients with and without NF2), which predispose individuals to nervous system tumors, including schwannomas, meningiomas, and ependymomas.[8,9,21,22,33,36,43,50,58,61,62,70,71,77] Abnormalities of the *NF2* gene are also found in malignant mesothelioma, tumors that are not typically associated with neurofibromatosis.[26,67] The presence of multiple meningiomas/schwannomas in an individual usually implies that there is a germ line mutation (with every cell in the body containing that mutation) or that the patient is a mosaic for a particular mutation (a proportion of cells within the individual's tissues harbor a mutation as a result of a postzygotic event).[14,29,30,45] Clinically, these patients are deemed to have classic NF2. Mild cases of NF2 usually result from a particular type of mutation (see *Genotype–Phenotype Correlation in NF2*), or may be the result of mosaicism (because the mutation is not present in every cell of the body). For obvious reasons, severe cases of NF2 are less likely to be the result of mosaicism.

Solitary meningiomas or schwannomas in an individual are normally the result of somatic mutation within the *NF2* gene in a single cell of neural crest origin. A "second hit" to knock out the remaining copy of the NF2 locus is then required to give this cell a growth advantage, which eventually leads to tumorigenesis. This second hit often consists of loss of one entire copy of chromosome 22 or a large proportion of the chromosome. This is usually the result of nondisjunction during mitosis in the cells containing the original point mutation. The term "loss of heterozygosity" describes the experimental findings in tumors that are missing one copy of a particular chromosomal region.[55] The "two hit" model of tumorigenesis involving tumor suppressor genes was first proposed by Knudson.[31]

It is likely that mutations in other, as yet unknown genes are necessary before a schwannoma or meningioma develops. One such candidate is the *P53* tumor suppressor gene, which has recently been shown to act synergistical-

Abbreviations used in this paper: HRS = hepatocyte growth factor–regulated tyrosine kinase substrate; NF1, NF2 = neurofibromatosis Types 1 and 2; Pak1 = p21-activated kinase; PIKE = phosphatidylinositol 3–kinase enhancer; PI3K = phosphatidylinositol 3–kinase; VS = vestibular schwannoma.

ly with the *NF2* gene (in conditional *NF2* mutant mice) in the development of malignant tumors of neural crest origin.[54] Furthermore, mutation/loss of the *NF2* gene is probably an early event in tumor development, with aberrations of genes on other chromosomes resulting in a more aggressive phenotype.[34]

Epidemiology and Clinical Features

The inherited syndrome NF2 is not a particularly common disease, with a birth incidence of approximately one in every 25,000 to 40,000 individuals.[11] As discussed earlier, however, the role of the *NF2* gene is not confined to patients with NF2 and has far more widespread implications in human disease. Mutations of the *NF2* gene occur frequently in sporadic tumors that are associated with the disease. For example, the majority of sporadic meningiomas harbor inactivating *NF2* gene mutations and/or chromosome 22 loss, as do a large proportion of sporadic schwannomas. These are among the most common tumors found in the central nervous system. Sporadic meningiomas comprise 20 to 25% of all primary intracranial neoplasms in the US (annual diagnostic incidence 8/100,000). Furthermore, results of autopsy studies support the suggestion that asymptomatic meningiomas are 10 times more common than clinically active ones. The incidence of VSs is approximately 1.4 per 100,000 individuals per year, and it is estimated that a VS will be diagnosed in one of every 1000 people during their lifetime.[12]

The diagnostic criteria for NF2 are well established and have been previously documented.[3,48] There have, however, been some slight modifications of these criteria in recent years. In most large series published to date, bilateral VSs develop in approximately 85 to 90% of patients with NF2 (VSs were previously called acoustic neuromas and are still frequently referred to by this name). A unilateral VS develops in another 5% of patients with NF2. Thus, up to 95% of patients with NF2 have at least one VS.

Schwannomas at other sites (including other cranial nerves, within the spinal cord, and on peripheral nerves) are also very common and are found in more than half of the patients in most studies.[39,40,53] Approximately 60 to 70% of patients with NF2 have at least one meningioma (intracranial and spinal). Posterior subcapsular lens opacities also develop in more than half of all patients with NF2. Ependymomas are seen in less than 5% of all patients with NF2, and less than 2% of patients with this disease have six or more café-au-lait macules, one of the diagnostic criterion for NF1 and sometimes a source of confusion between the two entities.

The NF2 *Gene*

The first clue to the localization of the *NF2* gene to chromosome 22 came from cytogenetic studies in sporadic meningiomas.[75] It was not until 1987, however, that a definitive localization to chromosome 22 (using genetic linkage studies in familial NF2) was established.[56] In a number of studies various authors have further refined the localization to 22q12.2, and concluded that NF2 appeared to be a genetically homogeneous disease.[47,59,72] The *NF2* gene was cloned in 1993 and found to be mutated/deleted in the majority of sporadic meningiomas and schwannomas. It comprises 17 exons and codes for a protein of 595 amino acids. The protein is closely related to the ERM proteins ezrin, radixin, and moesin. There are no hotspots for mutation in the *NF2* gene in patients with the disease, or in those with sporadic meningiomas or schwannomas. Furthermore, all possible types of mutation (missense, nonsense, frame-shift, splice-site, and various deletions) are found.

There is now overwhelming evidence that the *NF2* gene is a tumor suppressor gene. First, inactivating mutations in the *NF2* gene are found in the majority of patients with NF2 and in the sporadic tumors associated with this disease. Second, mice with targeted inactivation of the *NF2* gene specifically in Schwann cells develop schwannomas (the hallmark of NF2) and Schwann cell hyperplasia.[17] Furthermore, in mice that are heterozygous for an *NF2* mutation, numerous malignant tumors develop, including osteosarcomas at high frequency, and fibrosarcoma and hepatocellular carcinoma at a lower rate. There is also a growing body of evidence from cell biology experiments that the NF2 protein functions normally as a negative regulator of cell growth and proliferation (see later discussion).

Genotype–Phenotype Correlation in NF2

For many years it has been observed that the severity of NF2 was relatively preserved within individual families. That is, affected members within a family generally tended to experience symptoms of the disease at approximately the same age, and had a similar rate of progression with equivalent disability levels and similar numbers of tumors. It was clear clinically that a strong genotype–phenotype correlation existed for NF2. This is in contrast to some of the other genetic tumor syndromes (for example, NF1), in which there is often marked variation with regard to severity of disease within a given family. It is only since the identification of the *NF2* gene that it has been possible to prove this theory.[2,5,6,13,25–27,35,38,52,57,63,76]

Originally, NF2 was identified as either severe (Wishart) or mild (Gardner) disease. It is now apparent, however, that a more refined phenotype–genotype correlation exists. A milder NF2 phenotype (with an older age at onset and fewer tumors) is associated with missense mutations, splice-site mutations, and with certain large deletions. In addition, patients with mosaicism for any particular type of mutation tend to display a milder phenotype. One could further hypothesize that patients with mosaicism who harbor missense and splice-site mutations should have an even milder phenotype, although confirming this would require a very large series of patients. Severe disease is usually caused by protein-truncating alterations (such as frame-shift and nonsense mutations). One large study has recently shown that individuals with splice-site mutations in exons 1 to 5 of the *NF2* gene had more severe disease than those with splice-site mutations in exons 11 to 15. Phenotype–genotype correlations have now been documented for overall disease severity, for VSs, for cataracts,[4] and for non–eighth cranial nerve tumors (including intracranial meningiomas, spinal tumors, and peripheral nerve tumors). There are exceptions to this rule, however, and even in monozygotic twins the clinical course may not be identical.[6]

Biological Role of the NF2 *Gene*

The exact role of the *NF2* gene in tumorigenesis is not

entirely clear. Nevertheless, there are some very interesting clues to the possible mechanisms whereby loss of NF2 protein function may promote uncontrolled growth and proliferation. In the last 3 years alone, numerous reports have been published describing various cytoskeletal and signaling proteins that interact with and bind to the NF2 protein.

A number of these reports describe inhibition of the Pak1 by the NF2 protein.[1,20,23,25] This kinase belongs to a subgroup of serine/threonine kinases (the Group 1 Paks) that are known to be downstream effectors of Rac/cdc42. The Pak1 function is required for activation of stress-activated protein kinases by cdc42 and Rac1 as well as for transformation by activated forms of these guanosine 5′-triphosphatases. It has also been shown that activated forms of Pak1 induce rapid formation of membrane ruffles and focal complexes, and Pak1 is essential for both Ras transformation and NF1. The hypothesis is that the NF2 protein normally functions to inhibit Pak1 and therefore downregulates Rac/cdc42. This in turn would result in inhibition of Ras-induced malignant transformation. It has been shown in cell culture experiments that mutated *NF2* genes that lack the Pak1-inhibiting domain fail to suppress Ras transformation. Thus, Paks are probably bifunctional proteins, affecting both gene transcription (through a kinase cascade) and actin dynamics (by an unknown mechanism). A recent report suggests that the NF2 protein is normally involved in arresting cell growth at the G_1 phase with decreased expression of cyclin D1, inhibition of CDK4 activity, and dephosphorylation of pRB.[74]

Another interesting interaction exists between the NF2 protein and PIKE, which is a brain-specific guanosine 5′-triphosphatase that binds to PI3K and stimulates its lipid kinase activity. There are three isoforms of PIKE: PIKE-S (short form), PIKE-L (long form), and PIKE-A (alternative form). The PIKE-S and PIKE-L forms are the result of differential splicing, whereas PIKE-A results from the use of an alternative transcription initiation site. The NF2 protein binds to PIKE-L and abolishes its stimulatory effect on PI3K. The PI3K is a lipid kinase that generates phosphatidylinositol 3,4,5-trisphosphate, a second messenger that is essential for the translocation of Akt to the plasma membrane, where it is phosphorylated and activated. Activation of the PI3K/Akt pathway plays a pivotal role in fundamental cellular functions such as cell proliferation. In recent years, numerous alterations in this pathway have been described in a variety of human cancers. Thus, the involvement of the NF2 protein in an upstream component may prove to be a significant contributor to its tumor suppressor function.

The interaction of the NF2 protein with HRS warrants further discussion.[19,65,66,68] The NF2 protein binds (via its C terminus) to HRS, and it is postulated that this interaction may facilitate its ability to function as a tumor suppressor. Moreover, studies in schwannoma cell lines have shown that both the NF2 protein and HRS inhibit activation of signal transducers and activators of transcription. Recently, it has been shown in rat schwannoma cell lines that HRS can reduce the amount of total and active epidermal growth factor receptor. Thus, a possible direct consequence of NF2 protein inactivation may be the downregulation of epidermal growth factor receptor.

Other possible candidates for interaction with the NF2 protein include CD44, BII-spectrin, SCHIP-1, NHE-RF, and BI-integrin.[18,44,46,49,60,64] Furthermore, the NF2 protein colocalizes and interacts with adherens junction components in cell culture experiments. Knocking out the *NF2* gene in this environment results in an inability to undergo contact-dependent growth arrest and to form stable cadherin-containing adhesion junctions.[32] This may be an important factor in tumorigenesis.

A number of mouse models have been developed in an attempt to further elucidate the molecular biology of the NF2 protein.[17,41,42] Heterozygous *NF2* mutant mice suffer from various neoplasms that show inactivation of the wild-type copy of the *NF2* gene (in keeping with its function as a tumor suppressor gene). Nevertheless, in these mice there is no development of the characteristic tumors seen in human NF2. Furthermore, homozygous *NF2* mutant mice (homozygous NF2 knock-outs) are embryonic lethal and are therefore unhelpful as a model for NF2.

In an attempt to overcome the deficiencies of the heterozygous and homozygous *NF2* mutant mice, conditional *NF2* mutant mice were generated that had restricted homozygous *NF2* mutations in predominantly Schwann cell populations.[17] These "conditional" (Schwann cell) *NF2* knock-out mice show many similarities to patients with NF2; for example, Schwann cell hyperplasia, Schwann cell tumors, cataracts, and cerebral calcification. Significantly, however, they do not develop VSs or meningiomas, two of the hallmark features of NF2. The conditional knock-out mice have already provided important details regarding certain domains within the *NF2* gene (exon 2) and insight into how the NF2 protein may function in cell migration and differentiation. However, conditional *NF2* knock-out mice constitute only one piece of the NF2 puzzle, and extrapolation of results in rodents to human disease needs to be done cautiously.

CONCLUSIONS

Neurofibromatosis Type 2 has been extensively investigated and characterized from a genetics perspective over the last two decades. This has resulted in nearly 1000 research publications from numerous groups during this period. A new era in molecular biology is now unfolding in which researchers will characterize how genetic alterations in NF2 result in disruption of the regulation of different proteins and signaling pathways, resulting in tumor formation. It may be the end of one phase, but the biological interactions yet to be elucidated may prove even more exciting. Judging by the pace of the last few years, it may not be long before therapeutic strategies for NF2 and its tumors become a reality.

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