Mouse models of neurofibromatosis type I: bridging the GAP

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Neurofibromatosis type I (NF1) is an autosomal dominant disorder caused by mutations in the NF1 gene, leading to a variety of abnormalities in cell growth and differentiation, and to learning disabilities. The protein encoded by NF1, neurofibromin, has several biochemical functions and is expressed in a variety of different cell populations. Hence, determination of the molecular and cellular mechanisms that underlie the different NF1 symptoms is difficult. However, studies using mouse models of NF1 are beginning to unravel the mechanisms that underlie the various symptoms associated with the disease. This knowledge will aid the development of treatments for the different pathological processes associated with NF1.

Neurofibromatosis type I (NF1) is a common autosomal dominant disorder, affecting approximately one in every 3500 individuals (for reviews see [1,2]), and is one of the most common single-gene disorders influencing neurological function in humans [3]. Mutations in the NF1 gene result in abnormal cell growth and differentiation, with a variety of symptoms, typically including benign neurofibromas, hyperpigmentation of melanocytes, and hamartomas of the iris [1,2]. The benign neurofibromas can develop into malignant peripheral-nerve-sheath tumors (MPNSTs). Furthermore, in the brain, NF1 mutations can result in astrogliosis and astroglomas, and in learning disabilities that occur in 40–60% of patients with NF1 [4,5]. Visual-spatial function appears to be most compromised, although problems with language skills, executive function, attention and motor coordination are also common (for reviews see [4,5]).

Effects of NF1 mutations in humans and mice

The NF1 gene encodes a 250 kDa protein called neurofibromin, which has several known biochemical functions, including activation of the Ras GTPase [6–8], modulation of adenyl cyclase [9,10], and microtubule association [11] (Fig. 1). The human and mouse forms of neurofibromin are highly homologous (98% sequence similarity) [12], as are the promoter sequences of the gene, suggesting that both the biochemistry of the protein and the transcriptional regulation of the gene are conserved across species [12,13]. Although investigation of all the symptoms of NF1 in a single mouse model has proven difficult, the ability to dissociate some of the symptoms from others in particular mouse models (Table 1) has often been an advantage in studies of the disease. The effects of NF1 mutations in humans and mice show interesting parallels. For example, in mice and probably in humans, the complete loss of neurofibromin is lethal [14,15]. Also, NF1 mutations lead to tumor formation in both humans and mice. Aged mice heterozygous for a targeted disruption of the NF1 gene (NF1+/−) have an increased incidence of pheochromocytomas, and myeloid leukemias, two phenotypes observed in NF1 patients. Moreover, mice carrying NF1−/− mutations in particular cell populations develop neurofibromas [16,17] and mice that carry linked (cis) mutations in NF1 and p53 develop soft tissue sarcomas, namely MPNSTs [16,18]. Skin pigmentation problems have also been observed in mice and humans [19]. Chemically challenging NF1−/− mice with either a skin cancer initiator or a tumor promoter results in abnormalities in skin pigmentation and proliferation [20]. In addition, NF1 mutations result in abnormal brain function in both species. As described for NF1 patients [21], mice carrying...
Nf1 mutations can develop low levels of region-specific astrogliosis [22,23], and have learning deficits [24,25].

Mouse models of the cell growth and differentiation abnormalities in NF1

The Nf1 gene was cloned in 1990 [26–28] and soon afterwards mice were generated carrying a null mutation of this gene [14,15]. Homozygous Nf1-null mutant mice display heart malformation and hyperplasia of sympathetic ganglia, and die embryonically [15]. Mice heterozygous for the null mutation (Nf1+/−) are viable, but show an increasing incidence of tumor formation with age (especially phaeochromocytomas and myeloid leukemias) [14]. However, Nf1+/− mice do not develop the neurofibromas that are characteristic of NF1, which could indicate that loss of heterozygosity (LOH) is necessary for neurofibroma formation. To investigate this hypothesis, Cichowski et al. generated chimeric mice, composed partly of Nf1−/− cells, and these mice do develop neurofibromas [16]. However, several cell types are homozygous for mutant Nf1 in these mice and, hence, it is difficult to determine which cell type(s) are involved in the formation of neurofibromas. A recent study has addressed this problem by showing that mice with a homozygous null mutation specifically in Schwann cells (Nf1 flox/−; Krox20-cre) can develop neurofibromas [17]. This work also suggested that, in addition to homoygosity in Schwann cells, the development of neurofibromas requires heterozygosity in the surrounding cells [17]. Neurofibromas are usually benign, but they become malignant; for example, mice carrying linked mutations in Nf1 and p53 develop MPNSTs [16,18].

Skin pigmentations problems and epithelial carcinogenesis are commonly associated with NF1 [2]. Interestingly, Nf1+/− mice have been shown to have abnormal responses to skin wounding [19]. To determine if these mice are also more susceptible to pigmentation abnormalities or skin tumorigenesis, Nf1+/− mice in the C57/B6 genetic background, known for its resistance to chemical carcinogens, were challenged with either a skin-cancer initiator or a tumor promoter; even in the resistant genetic background, Nf1+/− mice developed skin pigmentation abnormalities and papillomas [20]. Furthermore, all of the papillomas had mutations in the H-Ras gene, suggesting that the decreased Ras GTPase activity in Nf1+/− mice had conferred increased susceptibility to carcinogenesis.

Astrogliosis is another symptom observed in NF1 patients [21]. Nf1+/− mice also develop astrogliosis in several brain regions, such as the nucleus accumbens, the periaqueductal gray, and the hippocampus. Interestingly, astrogliosis in the hippocampus (which is involved in spatial learning) only occurs in ~60% of the Nf1+/− mice [22]. This is consistent with the incidence of the spatial learning deficits in these mice [24], suggesting that the astrogliosis and the learning deficits could be associated. However, mice lacking exon 23a of Nf1 (Nf1 23a−/−) do not have astrogliosis, but do show learning deficits [25]. Interestingly, complete deletion of Nf1 specifically in glia [Nf1 GFAP conditional knockout (CKO)] [29], or specifically in neurons [Nf1 SynI knockout (KO)] [23], is sufficient to cause astrogliosis, indicating that disruption of neurofibromin in either cell type can contribute to this problem. Also, heterozygosity for Nf1 and p53, or Nf1 and Rb, confers a significant growth advantage to astrocytes (2.5-fold) [30]. Additionally, loss of both Nf1 and p53 result in blastoglioma formation [31]. Surprisingly, complete deletion of Nf1 specifically in astrocytes (Nf1 GFAP CKO) [29] is not sufficient to produce astrogliomas, a frequently observed benign tumor type in NF1 patients.

Mouse models of the learning disabilities associated with NF1

Despite the obvious differences between mice and humans, molecular and cellular cognitive studies have revealed remarkable similarities in the learning deficits caused by Nf1 mutations in the two species. First, the Nf1 mutation seems to affect some brain functions more than others. For example, visual-spatial learning [24], attention and motor coordination are all impaired, whereas various forms of learning, such as classical conditioning, seem to be intact. Second, only 40–60% of carriers of the mutation are affected, and remedial training can alleviate the learning deficits [4,24]. Third, the severity of the NF1 phenotype is affected by genetic variation, which exacerbates the

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Table 1. Mouse models of neurofibromatosis type I (NF1)
problem in NF1 patients without having a noticeable impact on normal siblings [24,32,33]. Consistent with this, it was shown that a heterozygous mutation of the N-Methyl-D-Aspartate receptor (NMDAR) increases the severity of the learning deficits in *Nf1* ^+/−^ mice without affecting learning in littermate controls [24]. Also, genetic background impacts on the incidence of learning disabilities in *Nf1* ^+/−^ mice [34]. These similarities suggest that mouse models could be useful for investigating the mechanisms that underlie the learning deficits associated with NF1.

The range of neurological symptoms associated with NF1 is vast and, hence, it is not clear whether the learning deficits associated with NF1 are caused by adult brain dysfunction, undetected tumors or neuro-developmental problems. However, results from recent work using mouse models have helped to clarify this matter. An interesting indication comes from studies of mice that carry a homozygous deletion of the alternatively spliced exon 23a of *Nf1* (*Nf1* ^23a−/−^) [25]. This exon encodes 63 base pairs (21 amino acids) within the GTPase activating protein (GAP)-related domain. Exclusion of exon 23a produces the type-I isofrom of NFI, whereas inclusion of this exon yields type II. The type-II isoform has a greater affinity for Ras, but lower GAP activity, than type I [35,36]. *Nf1* ^23a−/−^ mice are physically normal, and do not show any neural-crest development abnormality or increased tumor predisposition [25]. However, these mice do have learning impairments in spatial tasks (water maze and contextual discrimination) [25], much like the heterozygous null mutants [24,32]. These results show that the learning deficits can occur in the absence of tumor predisposition or neuro-developmental problems. Furthermore, mice lacking neurofibromin solely in neurons (*NF1* ^Hyp^/^KO^ without runty phenotype) show no increase in predisposition to tumor formation but do have learning deficits (R.M. Costa, PhD thesis, University of Oporto, 2002).

What, then, are the molecular alterations that underlie these learning disabilities? Neurofibromin has several known biochemical functions. For example, it has a GAP domain that accelerates the inactivation of Ras by stimulating its GTPase activity [6–8]. In addition, studies in *Drosophila melanogaster* and in mice have shown that neurofibromin modulates the activity of the rutabaga-encoded adenylyl cyclase (AC) [10,37], an enzyme crucial for cAMP signaling. Neurofibromin has also been shown to associate with microtubules [11], suggesting that it is involved in the regulation of multiple signaling pathways in the brain. All of these biochemical pathways are important for brain function, and have been implicated in synaptic plasticity and in learning and memory. However, the question remains as to which of these signaling pathways are crucial for the cognitive deficits associated with NF1.

Studies in *D. melanogaster* showed that homozygosity for mutation of *Nf1* causes associative learning deficits and that these deficits are dependent on the rutabaga-encoded AC [10]. Furthermore, the cAMP pathway is crucial for memory formation in a variety of vertebrate and invertebrate species [38], and AC activity is also impaired in cells from (lethal) homozygous mutant-*Nf1* mice [37]. However, in cells from heterozygous null mice, which have learning deficits, AC activity is indistinguishable from that of wild-type animals [37], whereas the Ras-controlled mitogen-activated protein kinase (MAPK) pathway is upregulated ([39], R.M. Costa, PhD thesis, University of Oporto, 2002). These data suggest that the ability of neurofibromin to regulate AC might be important for symptoms that require a loss of heterozygosity, whereas the Ras-GAP activity of neurofibromin might be crucial for phenotypes that are manifest in heterozygosity, such as learning deficits. Interestingly, however, the circadian phenotype of *Nf1* homozygous null mutants in *Drosophila* can be rescued by Ras mutations [40].

In humans, a mutation in the *NF1* gene that specifically abolishes the Ras-GAP function of neurofibromin, without affecting its ability to bind Ras, was shown to cause multiple NF1 symptoms, including cognitive dysfunction [41], suggesting that loss of the GAP function underlies the learning deficits.

In mice, recent studies showed that the spatial learning impairments in *Nf1* ^+/−^ mutants can be rescued by two different genetic manipulations that decrease Ras levels, indicating that increased Ras activity is the cause for the learning deficits [42]. In agreement, the learning deficits of *Nf1* ^+/−^ mice can also be reversed pharmacologically, using a farnesyl-transferase inhibitor (FTI), which indirectly reduces the amount of active Ras [42]. This FTI experiment also indicates that the learning deficits of *Nf1* mutant mice can be reversed in the adult, a finding of crucial importance for the development of treatments for the learning disabilities associated with NF1. In addition, *Nf1* ^23a−/−^ mice, which lack the alternatively spliced exon 23 (which modulates the GAP activity of NFI) have learning disabilities [25].

Several other genetic and pharmacological studies have substantiated the role of Ras signaling in synaptic plasticity, and learning and memory [43–45].

Taken together, these studies suggest that either abnormally high or low Ras activity can disrupt learning, indicating that precise Ras modulation by neurofibromin is crucial for learning and memory. It will be important to determine which Ras effectors (Fig. 1) are crucial for the learning deficits associated with NF1. Also, in light of the *Drosophila* studies described above [10,37], it will be important to determine whether, in mammals, regulation of the cAMP pathway by neurofibromin plays a role in the occurrence of learning disabilities. If so, it would be interesting to observe whether different behavioral impairments can be attributed to abnormalities in either the cAMP or the Ras pathways, or whether these two pathways interact during learning in a manner regulated by neurofibromin.

The results of the experiments described above suggest that the learning deficits associated with NF1 result from adult brain dysfunction caused by increased Ras activity. Therefore, it is important to understand how *Nf1* mutations, and the subsequent Ras hyperactivity, alter neuronal physiology. Activity-dependent synaptic modifications in neuronal networks are thought to mediate learning. Long-term potentiation (LTP) is a widely studied...
experimental model of the synaptic plasticity mechanisms thought to underlie learning and memory [46]. Nf1+/− mutant mice show learning deficits in the hidden version of the water maze, a spatial navigation task that is dependent on hippocampal function [47]. In humans, the hippocampus seems to be crucial for the acquisition of new declarative memories [48,49]. Electrophysiological studies in the CA1 region of the hippocampus of Nf1+/− mice showed that LTP is impaired when induced using a theta-burst stimulation (TBS) protocol [42], which mimics the in vivo activity of hippocampal neurons during exploratory behavior [50]. Further electrophysiological analysis showed that the Nf1+/− mice have increased y-aminobutyric acid (GABA) inhibition in the hippocampus, and suggested that this increased inhibition is the cause of the LTP deficits [42]. Remarkably, as with the learning deficits, both the increased GABA inhibition and the LTP deficits of the Nf1+/− mutant mice can be reversed by manipulations that decrease Ras activity [42].

Ras has been shown to modulate chloride currents in adrenal gland cells [51]. The data described above suggest that Ras might also modulate inhibitory currents in the central nervous system. Interestingly, whereas neurofibromin affects LTP through modulation of GABA-mediated inhibition, H-Ras [52] and even other GAPs, such as Syn-GAP [53], have been implicated in LTP via their modulation of excitatory neurotransmission. Neurofibromin is also localized at excitatory synapses and interacts with the NMDAR in a large post-synaptic complex [54]. Furthermore, in excitatory synapses, Ras mediates synaptic delivery of AMPA (α-amino-3-hydroxy-5,7-methylisoxazole-4-propionic acid) receptors during LTP [55]. Hence, neurofibromin might modulate excitatory as well as inhibitory synaptic function.

The role(s) of neurofibromin and Ras at inhibitory synapses are not yet known. Pre-synaptically, Ras could as well as inhibit synaptic function.

Conclusions

Studies employing mouse models of NF1 have been crucial in investigating the pathophysiology of different aspects of the disease, such as problems with cellular differentiation, tumor formation and learning. These studies have been essential in identifying the cell types, the cellular processes and the molecular pathways that are altered by Nf1 mutations. This knowledge might have important implications for the development of treatments for NF1.

References


7 Xu, G.F. et al. (1990) The catalytic domain of the neurofibromatosis type 1 gene product stimulates ras GTPase and complements ira mutants of S. cerevisiae. Cell 63, 835–841


13 Hajra, A. et al. (1994) DNA sequences in the promoter region of the NF1 gene are highly conserved between human and mouse. Genomics 10, 649–652


15 Brannan, C.I. et al. (1994) Targeted disruption of the neurofibromatosis type-1 gene leads to developmental abnormalities in heart and various neural crest-derived tissues. Genes Dev. 8, 1019–1029


19 Atti, R.P. et al. (1999) The NF1 tumor suppressor regulates mouse skin
wound healing, fibroblast proliferation, and collagen deposition by fibroblasts. J. Invest. Dermatol. 112, 835–842
22 Rizvi, T.A. et al. (1999) Region-specific astrogliosis in brains of mice heterozygous for mutations in the neurofibromatosis type 1 (NF1) tumor suppressor. Brain Res. 816, 111–123
31 Reilly, K.M. et al. (2000) NF1;Trp53 mutant mice develop glioblastoma with evidence of strain-specific effects. Nat. Genet. 26, 109–113
50 Larson, J. et al. (1986) Patterned stimulation at the theta frequency is optimal for the induction of hippocamal long-term potentiation. Brain Res. 368, 347–350
52 Manabe, T. et al. (2000) Regulation of long-term potentiation by H-Ras through NMDA receptor phosphorylation. J. Neurosci. 20, 2504–2511
57 Yamagata, Y. et al. (2002) Bidirectional changes in synapsin I phosphorylation at MAP kinase-dependent sites by acute neuronal excitation in vivo. J. Neurochem. 80, 835–842
60 Lu, Y.M. et al. (2000) Calcineurin-mediated LTD of GABAAergic inhibition underlies the increased excitability of CA1 neurons associated with LTP. Neuron 26, 197–205

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