Neurofibromatosis Type 1: Involvement of NF1 Mutations in Nervous System Tumours and Learning and Cognitive Dysfunction in this Disorder

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Abstract

Neurofibromatosis type 1 (NF1) is a disorder caused by mutations in a single gene. These mutations result in neurological dysfunction including learning disabilities in children and cognitive impairment in adults. Individuals with NF1 often develop benign and sometimes malignant neoplasms of the brain and peripheral nerves. The underlying gene responsible for these defects is the NF1 gene, which is located on chromosome 17q11.2. The NF1 gene encodes a protein called neurofibromin. One known function of neurofibromin is to inhibit another gene called Ras from sending signals to other genes and proteins. Mutations of the NF1 gene disrupt neurofibromin function, impairing its ability to inhibit Ras. This lack of inhibition of Ras, which is equivalent to its aberrant activation, underlie the basis of tumour formation in NF1. Although most tumours are benign, some may acquire further mutations that lead to malignant transformation. Studies in mice are now beginning to suggest that aberrant activation of Ras by NF1 mutations also promotes inhibition of synapse function by the neurotransmitter gamma-aminobutyric acid (GABA), and impairs a process called long-term potentiation. Such events may account for the learning and cognitive deficits seen in humans. Unusual terms in the text of the paper are explained in a glossary.

Neurofibromatosis 1 (NF1) is one of the most common genetic disorders that result in nervous system dysfunction. This dysfunction manifests as specific learning disabilities and cognitive impairments that do not disappear and that continue to cause problems throughout one's life (i.e.,

lifetime morbidity). One almost inevitable consequence of NF1 affliction is the development of nervous system tumours as the result of loss of function of the NF1 gene (Gutmann, 2002). Research over the past decade has provided detailed insight into how NF1 dysfunction results in the molecular, cellular, physiological, and phenotypical abnormalities seen in affected individuals. The development of mouse models that exemplify some of the clinical features of NF1, and the application of therapies that are targeted to NF1-associated tumours, are guiding an exciting era of clinical and basic science that offers the hope of improved, rationally based treatments for NF1 in the foreseeable future. The goal of this paper will be to describe some of the common nervous system tumours that occur in patients with NF1, highlight important progress that has been made in terms of understanding mechanisms underlying tumourigenesis and cognitive impairments observed in NF1 patients, review the molecular biology of the NF1 gene and the different types of mutations that contribute to NF1 gene dysfunction, discuss current treatment options, and conclude with questions that remain to be addressed in NF1 research.

Neurofibromatosis Type 1

NF1 is the most common monogenic (single gene) disorder affecting the human nervous system. Approximately 1 in 3,000 individuals world wide have NF1, regardless of race, gender, or ethnic background (Arun & Gutmann, 2004; Young, Hyman & North, 2002). It is transmitted in an autosomal dominant fashion and affects males and females almost equally. NF1 affects both skin and nervous tissue and is considered a neurocutaneous disorder that manifests as café-au-lait spots, skinfold freckling, cutaneous (skin) neurofibromas, and iris hamartomas. It is also considered a multi-system disorder, affecting the eyes, skeleton, blood vessels, endocrine system, and the central nervous system (CNS) (Young et al., 2002). Table 1 summarizes the National Institutes of Health diagnostic criteria for NF1. Mutations in the NF1 gene cause abnormalities in cell growth and differentiation and lead to a variety of learning disabilities.

With respect to CNS abnormalities, NF1 patients in pediatric populations often exhibit specific learning disabilities, Attention Deficit Disorder, and hyperintense lesions in a form of brain imaging called T2-weighted brain magnetic resonance imaging (MRI) (Arun & Gutmann, 2004). Cognitive deficits and academic learning disabilities account for the most common neurological complication of NF1 in children, and can be responsible for significant lifetime morbidity (North, Hyman & Barton, 2002). Deficits in

spatial ability, higher order (executive) function, expressive and receptive language skills, and behavioural and psychosocial problems have also been documented in NF1 individuals (North et al., 2002).

Table 1. National Institutes of Health diagnostic criteria for NF1[*]

· Six or more café-au-lait macules over 5 mm in greatest diameter in prepubertal individuals and over 15 mm in greatest diameter in postpubertal individuals

· Two or more neurofibromas of any type or one plexiform neurofibroma

· Freckling in the axillary or inguinal regions.

· Optic glioma

· Two or more Lisch nodules (iris hamaratomas)

· A distinctive osseous lesion such as sphenoid dysplasia or thinning of long bone cortex with or without pseudoarthrosis

· A first-degree relative (parent, sibling or offspring) with NF1 by the above criteria

[*]An individual is considered to have NF1 when two or more of the above features above are met. However, these diagnostic criteria are not sufficient for establishing the presence of NF1 in small children without a family history of the disease (DeBella, Szudek & Friedman, 2000; Friedman, 2002; Szudek, Evans & Friedman, 2003; von Deimling, Krone & Menon, 1995).

NF1 Associated Nervous System Tumours

In addition to cognitive impairments, both children and adults with NF1 often develop benign and malignant tumours involving the brain and peripheral nerves. Tumour formation in these individuals results from mutational inactivation of the NF1 gene, located on chromosome 17q11.2 (Viskochil, 2002). The NF1 gene has been suggested to function as a tumour suppressor gene. This means that when the gene is active, the protein that is produced by the gene prevents tumours from forming. As with other inherited tumour syndromes and tumour suppressor genes, NF1 individuals harbour one mutated non-functional NF1 gene (as a result of a germline mutation or deletion), and one normal functional NF1 gene. If the normal NF1 gene becomes mutated or is lost from a cell by deletion (a process called loss of heterozygosity) then the lack of production of the protein normally expressed by the NF1 gene, namely neurofibromin, results in aberrantly elevated levels of intracellular Ras activity and tumour formation. Thus, one function of neurofibromin is to inhibit Ras activity (see below)

(Arun & Gutmann, 2004; Woods et al., 2002). Because the inhibition of Ras activity suppresses tumour formation, it is known as an oncogene. Box 2 summarizes symptoms that would lead a clinician to suspect a tumour in NF1.

Table 2. Symptoms that would lead a clinician to suspect a tumour in NF1[*]

· vision loss

· involuntary movement of eyeball

· outward bulging of the eye (painless proptosis)

· convulsions

· pain associated with affected peripheral nerves

[*] *These symptoms usually result from progressive growth of a mass that invades and presses on the optic nerve and adjacent structures (Astrup, 2003;Thiagalingam, Flaherty, Billson & North, 2004).*

Neurofibromas

Neurofibromas are the most prevalent tumours that occur in individuals with NF1 and represent the second most common of all peripheral nerve tumours (Friedman, 2002). A neurofibroma is a benign tumour that is composed predominantly of Schwann cells, but it may also include fibroblasts, pericytes, smooth muscle cells and mast cells, all of which may contribute to the growth of involved nerves (Woods et al., 2002).

Malignant periperal nerve sheath tumour

Although dermal (skin) neurofibromas are benign and do not progress to malignancy, intraneural plexiform neurofibromas that arise from larger and deeper nerves can undergo malignant transformation, giving rise to malignant peripheral nerve sheath tumours (MPNSTs) (Woods et al., 2002). Although previous studies have shown that 3-5% of NF1 patients progress to develop MPNSTs, more recent reports indicate a much higher frequency, ranging between 8-13% (Arun & Gutmann, 2004). MPNSTs in NF1 patients occur at a relatively earlier age of onset than in sporadic cases of MPNSTs. Characterization of the spectrum of mutations in MPNST has revealed that large NF1 genomic deletions carry a significantly elevated lifetime risk for developing MPNST, raising the intriguing possibility that this particular type of NF1 mutation may serve as a marker or risk factor that identifies a subset of patients with NF1 who may develop MPNST (Arun & Gutmann, 2004).

Optic pathway gliomas

Optic pathway gliomas (OPGs) are the second most predominant tumours that occur in approximately 15% of children with NF1 (Arun & Gutmann, 2004). Although OPGs account for only 2-5% of all brain tumours in childhood, as many as 70% are associated with NF1 (Friedman, 2002; Listernick, Charrow & Gutmann, 1999). These tumours arise within the first four years of life; however only one-third to one-half of patients with OPGs develop progressive clinical symptoms that require treatment (Arun & Gutmann, 2004; Friedman, 2002). OPGs in NF1 patients are usually low grade-pilocytic astrocytomas that arise in the optic nerve and chiasm, hypothalamus, brainstem, and cerebellum (Listernick et al., 1999; Rosser & Packer, 2002).

Brain stem tumours

Two studies have reported the occurrence of brain stem tumours in patients with NF1. These tumours generally arise between 8-10 years of age and like OPGs, they are relatively benign. The medulla is the most common tumour site in patients with NF1, accounting for 82% of cases, while the pons is the predominant tumour site in individuals not afflicted with NF1. In the majority of NF1 associated brain stem tumours, the patients rarely require treatment interventions (Listernick et al., 1999; Molloy et al., 1995; Pollack, Shultz & Mulvihill, 1996).

NF1 associated astrocytomas

Sporadic high-grade astrocytomas do not usually exhibit NF1 gene inactivation or neurofibromin loss; however, recent reports have suggested that patients with NF1 are at a significantly increased risk of developing astrocytomas (Gutmann et al., 2003; Listernick et al., 1999). For instance, later-onset NF1-associated astrocytomas, unlike histologically identical sporadic astrocytomas, have been shown to exhibit NF1 inactivation, supporting a direct association with NF1. Moreover, some of these astrocytomas harbour homozygous NF1 deletions as well as additional genetic perturbations that frequently occur in sporadic high-grade astrocytomas (such as those resulting from TP53 mutations or CDKN2A/p16 deletions) (Gutmann et al., 2003).

Mechanisms Underlying Tumourigenesis

Rationale for using mouse models

Mouse models have been very useful in delineating the molecular mechanisms that underlie tumour formation associated with NF1. The products of the mouse and human NF1 genes, namely mouse and human neurofibromin, are highly homologous. Their amino acid sequences are approximately 98% identical. The region of a gene that regulates its transcription to mRNA is called the promoter region. This region of the NF1 gene also is very similar in mouse and human. This striking conservation of sequence at the DNA level suggests that processes that regulate mRNA production from the NF1 gene and the biochemistry of neurofibromin are conserved across species (Bernards, Snijders, Hannigan, Murthy & Gusella, 1993; Costa & Silva, 2002). NF1 gene mutations in both humans and mice exhibit similar phenotypes (Bernards et al., 1993; Costa & Silva, 2002). In the mouse, complete removal of the NF1 genes by genetic manipulation is embryonically lethal, and the occurrence of two identical NF1 mutations results in hyperplasia (overgrowth) of astrocytes which may lead to tumour formation. Tumours have also been shown to occur in offspring from NF1 deficient mice that are crossed with p53 heterozygous (+/-) or p53 deficient (-/-) mice. In addition, loss of one NF1 allele is sufficient to cause abnormal brain function in mice (Costa & Silva, 2002). These characteristics validate the use of mice as a model system to study NF1.

Constitutive activation of mitogenic signal transduction

The use of NF1 mouse models and cultured cell lines have permitted detailed investigation of the mechanisms underlying NF1 mediated tumourigenesis. These studies have collectively demonstrated that the protein product of the NF1 gene, neurofibromin, functions as a tumour suppressor by inhibiting cell growth and proliferation. Specifically, neurofibromin suppresses the activation of the protein Ras, which when aberrantly activated, can lead to tumourigenesis. Indeed, abnormal activation of Ras has been shown to not only occur in nervous system neoplasms, but in other tumours such as those derived from the epithelium (carcinomas), and other human cancers as well (Webb, Van Aelst, Wigler & Woude, 1998; White et al., 1995;Woods et al., 2002).

Ras is a member of the guanosine triphosphate (GTP)-binding proteins, that are also known as G proteins. G proteins are signal transducing proteins; that is, they transmit intracellular signals from cell surface receptors to the

nucleus to regulate transcription of genes involved in cell growth and differentiation. Aberration of G protein signalling is fairly common in nervous system tumours such as NF1, neurofibromas, MPNSTs, and astrocytomas (Woods et al., 2002). Activation of Ras can occur by a number of different processes: abnormal overexpression of the activated form of Ras, activation of growth factor receptors that activate Ras, and, with relevance to neurofibromatosis associated neurofibromas and MPNSTs, loss of expression of neurofibromin, a major inactivator of Ras. Activation of Ras by any of these mechanisms activates a number of pathways involved in various aspects of tumourigenesis, such as cellular proliferation and migration, and expression of angiogenic factors. As Ras is a critical mediator of the mitogen activated protein kinase (MAPK) signalling cascade (a signalling pathway that when activated induces cells to grow and divide), activation of this pathway is thought to be the underlying basis for tumour formation in people with NF1.

Mechanisms Underlying Cognitive Deficits

As described above, mutations in the NF1 gene result in abnormal cell growth and often tumourigenesis. However, the majority of research in this area has focused on mechanisms of tumourigenesis resulting from NF1 gene dysfunction. The molecular and cellular mechanisms underlying cognitive deficits in NF1 are areas of investigation that have only recently garnered attention.

Just as there are relative similarities between mice and humans with regard to molecular mechanisms underlying tumourigenesis by disrupting NF1 gene function, there are also striking similarities between the learning deficits caused by NF1 mutations in mice and humans. For instance, in both species NF1 mutations appear to impair similar brain functions such as visuospatial learning, attention, and motor co-ordination (Costa & Silva, 2002).

One criticism of this approach, however, is the validity of the leaning tasks used in these studies. For instance, in these studies, can the "Judgement of Line Orientation Test" used in mice to assess cognitive learning and behavioural tasks be equated to cognate learning tasks in humans? In other words, can the tests that are used to assess learning impairments in these animals be equated to the type of leaning tasks performed by humans? Furthermore, it is important to note that some of these deficits could be secondary to developmental abnormalities and other neurological problems, such as poor motor coordination and attentional deficits, and not to NF1 gene dysregulation per se. Hence, with regard to cognitive impairments associated with NF1 gene dysregulation, findings must be interpreted with cautious optimism.

Molecular

As noted above, one well characterized function of neurofibromin is to inhibit activation of Ras by virtue of its guanosine triphosphatase-activating domain (Costa & Silva, 2002). It has been shown in humans that a specific NF1 gene mutation that abolishes the Ras-guanosine triphosphate-activating function of neurofibromin, without affecting its ability to bind Ras, causes NF1 symptoms that include cognitive dysfunction (Costa & Silva, 2002). This suggests that loss of the guanosine triphosphatase-activating protein function may underlie the learning deficits. This hypothesis is supporting by findings in NF1 +/- mutant mice that exhibit spatial learning impairments; these can be rescued by pharmacological and genetic manipulations that decrease Ras levels and activity (Costa & Silva, 2002; Costa et al., 2002). (NF1 +/- mutant mice lack one functional NF1 allele; this impairs the Ras-guanosine triphosphatase-activating function of neurofibromin in these animals.) Pharmacological inhibition of Ras in these studies was achieved by the farnesyl transferase inhibitors, which targets the enzyme farnesyl trasferase and prevents it from adding a farnesyl molecule onto Ras, a process that is required for Ras to become activated. Collectively, these studies suggest that elevated Ras activity can disrupt learning, and that precise Ras modulation by neurofibromin is critical.

Figure 1. Schematic representation of the NF1 gene product, neurofibromin, depicting regions involved in regulating Ras signalling (GRD) and residues required for microtubule binding.
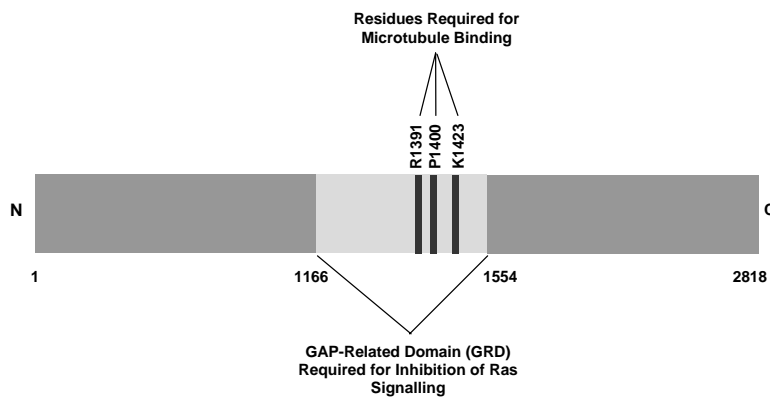
Modified from (Gutmann & Collins, 1993). GAP: Ras-GTPase activating protein.

Another less well characterized property of neurofibromin is that it can directly bind to and associate with microtubules, and that specific mutations to the NF1 gene disrupt this interaction in vitro (Figure 1) (Li, Cheng, Gutmann & Mangoura, 2001). Although the protein domains required for, and mediating the interaction, have been characterized in great detail, the functional importance of this interaction is unclear. One intriguing mechanism that could contribute to the cognitive deficits seen in NF1 is that mutation to the NF1 gene results in disruption of the association between neurofibromin and microtubules in NF1 patients which could result in

Figure 2. Schematic representation of a hypothetical model involving microtubules in NF1 pathobiology.
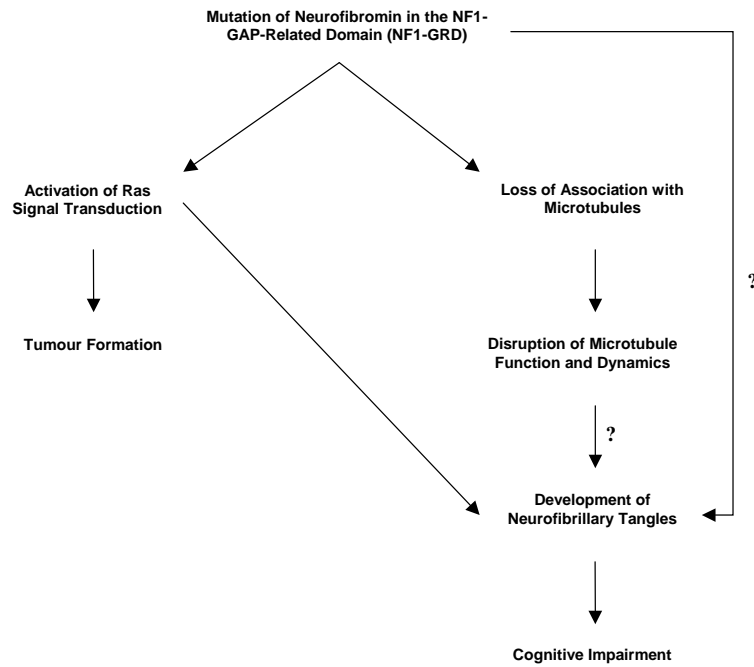
It is unknown whether NF1 gene mutations affect the development of neurofibrillary tangles. In this model, mutations in the NF1 gene that disrupt the ability of neurofibromin to bind microtubules may promote development of neurofibrillary tangles, leading to cognitive impairments. The ability of neurofibromin to associate with the microtubule binding protein, tau, and its phosphorylation status in NF1 patient specimens has not been documented.

perturbation of microtubule function and dynamics, that could lead to formation of neurofibrillary tangles (NFTs) similar to those seen in other neurodegenerative disorders in which cognition and learning are affected (Figure 2). Although it has been shown that elevated expression of Ras is an early event in Alzheimer disease and precedes the formation of NFTs that are found in brain neurons in this disorder (Gartner, Holzer & Arendt, 1999), there are currently no studies that have systematically addressed the presence or absence of NFTs at the light or electron-microscopic levels in NF1 mice or patient specimens. In Alzheimer disease, NFTs contain hyperphosphorylated tau molecules (abnormal addition of phosphate groups onto the protein tau, which normally binds to microtubules in cells). In this context, it would also be of interest to determine if tau associates with neurofibromin, something that could be readily tested by 2-colour confocal microscopy and co-immunoprecipitation experiments. These experimental techniques are commonly used to detect protein-protein interactions.

Cellular

How do the molecular changes described above alter neuronal physiology that translates into learning deficits? Insights into this question have come from electrophysiological studies using NF1 +/- mutant mice that demonstrate significant impairment of long-term potentiation (LTP, a widely studied experimental model of synaptic plasticity mechanisms, thought to underlie learning and memory when induced with a theta-burst stimulation protocol) (Costa & Silva, 2002). These mice demonstrate increased ?-aminobutyric acid (GABA) inhibition in the hippocampus responsible for the LTP deficits. This means that activation of GABA in hippocampal neurons of NF1 +/- mice is suppressed to a greater extent than in the hippocampal neurons derived from NF1 +/+ mice. Taken together, the molecular and cellular data argues in favor of a model whereby NF1 mutations result in increased Ras activation, which leads to abnormally elevated GABA-mediated inhibition, and that this enhanced inhibition depresses LTP which manifest as learning deficits. This model could be greatly strengthened if it could be shown that in these mice, synaptic density at the ultra-structural level (i.e. electron-microscopy), is reduced relative to controls. In other words, could it be that the total number of synapses formed in NF +/- mice is reduced as a result of increased GABA inhibition? To date, not much work has been done to systematically and specifically address this question in NF1 mice and NF1 patient samples.

Genetics of Neurofibromatosis 1

Molecular biology of the NF1 gene

The NF1 gene was identified in 1990 by positional cloning in families with multiple affected individuals (reviewed in Viskochil, 1999). The condition was genetically mapped to chromosome 17q11.2. The NF1 gene spans approximately 350 kb of genomic DNA and is composed of 60 exons. There are three alternatively spliced variants, with the processed full length form being 11 to 13 kb length with a 3.5-kb 3' untranslated region. The NF1 promoter is embedded in a CpG-rich region that lacks transcription factor bind sites. It shares high sequence similarity with the mouse NF1 promoter (Viskochil, 1999; Viskochil, 2002).

The NF1 locus harbors a number of polymorphic markers such as: single nucleotide polymorphisms located in exons 5 and 13, and in the 3' untranslated region; an Alu tetranucleotide repeat on intron 27b; and a Ta subclass L1 insertion on intron 30. In addition, dinucleotide repeats have been found throughout the gene, which have been useful for genotype analysis (Viskochil, 2002).

Mutational aspects of the NF1 gene

Of the gamut of mutations known to occur on the NF1 gene, mutations in the promoter region are not common, and hypermethylation of the promoter is not a common event in the inactivation of the normal NF1 allele in NF1 related tumours (Viskochil, 1999). Mutations in the NF1 gene predict inactivation and haploinsufficiency of neurofibromin. One mechanism by which this occurs is by the loss or gain of a small direct repeat sequence within the open reading frame (the region of the gene that codes for the neurofibromin). In this way, replication errors occur which are likely attributable to slippage of the DNA replication machinery during DNA synthesis, or the deletion of a sequence that has inverted repeats at the ends, and is thus looped out as a hairpin and skipped in replication. The frequency of this type of mutation in NF1 has been estimated at 20-30% and usually results in a deletion rather than an insertion (Thomson, Fishbein & Wallace, 2002).

Another class of mutations that occurs in NF1, are those that affect RNA splicing. Here, the consensus splice donor or acceptor sequence is altered and results in omission of the nearby exon. Such mutations usually occur in about 20-35% of NF1 cases, however, since many mutations have been

reported at the DNA level without RNA level analysis, it is likely that splicing related mutations represent a larger than expected proportion of mutations (Ars et al., 2000; Messiaen et al., 1999; Messiaen et al., 2000).

Another mutation mechanism that is observed in NF1 is point substitutions, which are single base changes that are not silent. Point mutations that create stop codons occur with a frequency of between 30-38% in NF1, whereas other point changes in the open reading frame that alter the amino acid sequence account for approximately 5-10% of NF1 mutations (Thomson et al., 2002). The manner by which these point mutations result in pathogenesis is unclear; however, it has been suggested that these substitutions affect RNA splicing (Thomson et al., 2002).

Intragenic deletions and insertions represent another type of mutation in NF1, and these account for the rarest type, accounting for less than 5% of all NF1 cases. One corollary is that these types of mutations are difficult to detect with current methods, and therefore may be under-represented (Lazaro et al., 1995; Upadhyaya et al., 1990). Another rare class of mutations that disrupt the NF1 gene are those that are caused by chromosome translocation or inversion. However to date, only a few cases of this type have been reported (Thomson et al., 2002).

The last class of mutations, which account for 2 to 10% of all NF1 cases, are those in which large deletions occur in the NF1 gene as well as in surrounding sequences. There is a relatively common genomic site for deletion breakpoints in NF1 microdeletion syndrome, which occur mostly as the result of recombination between repetitive elements that are approximately 1.5 Mb apart (Dorschner, Sybert, Weaver, Pletcher & Stephens, 2000; Viskochil, 2002). This event is usually maternal in origin and results from unequal crossing over in meiosis I. Three embedded genes and nine contiguous genes have been identified in the most common deletion. Two sites that demonstrate frequent recombination are 2-kb paralogous sequence blocks, one being approximately 400 kb upstream of NF1, and the other being approximately 700 kb downstream of NF1 (Dorschner et al., 2000; Jenne et al., 2001; Lopez Correa et al., 1999; Lopez-Correa et al., 2001). Patients harbouring these mutations are at increased risk for early and greater tumour burden as well as mental deficits and dysmorphism (Thomson et al., 2002).

Therapeutic Strategies

Neurofibromatosis 1 is not usually a fatal disorder; however, affected individuals often face a lifetime of morbidity and disfigurement.

Management of NF1 has focused predominantly on anticipatory guidance, genetic counselling, and symptomatic treatment of specific lesions. Treatment of the associated nervous system tumours such as neurofibromas has entailed surgical resection, but this approach has often been limited by the inaccessibility of many of the tumour foci, the potential for complications thereof, and the likelihood for some tumours to recur (Korf, 2002).

Non-surgical treatment of NF1 is currently aimed at targeting the associated tumours. Therefore, early and accurate diagnosis of the tumour and prompt initiation of a treatment regimen is critical. Targetting the Ras pathway would appear to be the ideal strategy and clinical trials for farnesyl protein transferase inhibitor are currently in place, with results still pending. One can speculate that by inhibiting oncogenic Ras signal transduction, there may be an accompanied improvement in cognitive and executive functions, as discussed above. Other drug treatments that do not target the Ras signaling pathway specifically, but that inhibit fibroblast proliferation, angiogenesis, and promote apoptosis are also being tested in clinical trials. Although uncommon, and depending on the type of tumour, radiation therapy also remains an option for NF1 patients (Korf, 2002).

Outstanding Questions and Future Directions

Although significant progress has been made toward understanding the structure and function of the NF1 gene, there is still yet a great deal that remains to be learned. For instance, the role of neurofibromin in suppressing Ras signal transduction is well characterized in molecular detail; however, its involvement in other signalling pathways that regulate cellular growth and proliferation (such as the protein kinase A and phosphatidylinositol 3'-kinase pathways) is unclear. Moreover, the finding that neurofibromin binds to the cytoskeleton suggests that in addition to playing a role in signal transduction, there may be additional adapter (scaffolding) functions of neurofibromin that regulate distinct cellular processes. Furthermore, the functional importance of the different molecular forms of NF1 that result from the single NF1 gene (isoforms) needs to be addressed systematically. Finally, the variable expressivity of the NF1 phenotype in patients suggests that there are likely additional modifier genes that influence the pathobiology of this disorder. Genetic studies using mice will no doubt be critical to identify such genes, and may assist in the development of additional therapeutic targets (Finkelstein, 2002).

One intriguing question that arises from this analysis is that why, if there is mutation that affects only one gene, is there a spectrum of nervous system

tumours that result? Why for example, do we not see one particular type of tumour exclusively in NF1 patients, but instead see a spectrum of different ones? It might be that the different types of mutations result in the production of mutant forms of neurofibromin which may have different oncogenic properties. For instance, some mutant proteins may have disrupted catalytic function but may retain their protein binding properties. Alternatively, other mutant proteins may activate other mitogenic signalling pathways that could result in uncontrolled cell proliferation. Future studies should be aimed at deciphering how mutations to NF1 affect the properties of neurofibromin and test whether these proteins confer transforming capabilities.

Acknowledgements

Glossary

Angiogenesis- The process in which new blood vessels are formed. This normally occurs during development of the embryo, but can also occur abnormally around malignant tumours.

Apoptosis- Physiological (as opposed to pathological) cell death that occurs by an active process, requiring metabolic activity by the dying cell. Hallmarks of cells undergoing apoptosis include shrinkage of the cell and cleavage of the DNA into fragments. It is also referred to as "programmed cell death".

Astrocytoma- A neuro-ectodermal tumour arising from astrocytes.

Autosomal dominant- A type of genetic inheritance in which an individual carries a normal functioning gene on one allele, and a mutant gene on the other. An autosomal dominant gene can be inherited from a single affected parent where both males and female offspring have an equal chance of inheriting the gene.

Benign- A tumour that is made up of cells that have no potential to spread and grow in another location in the body.

Brain stem- The part of the brain that is continuous with the spinal cord and comprises the medulla oblongata and pons and midbrain, as well as parts of the hypothalamus.

Café-au-lait spots- Pigmentary abnormalities of the skin that are light brown in colour.

Chromosome inversion- A type of mutation in which part of the genetic sequence of a chromosome is reversed.

Chromosome translocation- Chromosomal abnormalities that occur when chromosomes break and the fragments rejoin to other chromosomes.

Co-immunoprecipitation- An experimental procedure that is used to determine if two different proteins interact. An antibody specific to the protein of interest is added to a cell extract. Then the antibody-protein complex is pelleted usually using protein-G sepharose which binds most antibodies. If there are any protein/molecules that bind to the first protein, they will also be pelleted. Identification of proteins in the pellet can be determined by western blot (if an antibody exists) or by sequencing a purified protein band.

Confocal microscopy- A technique used to obtain high quality non-invasive sections of fluorescent-labeled specimens and visualize structures within cells. One advantage of this technique is that it allows for discrimination of several fluorescence markers on the same specimen and thus, the concurrent detection of several intracellular structures labeled by specific probes can depict a comprehensive view of the functional organization of cells and tissues. Digital images produced by confocal microscopes are ideally suited for quantitative analysis. Pixel histograms give an account of the pixel value probability density and we can extend statistical properties by estimating joint probabilities linking fluorescence intensity distributions of multiply-labeled specimens, which can provide information of relative proximities between labeled structures.

CpG-rich region- Region of genomic DNA rich in the dinucleotide cytosine-guanine bases. Methylation of the cytosine in the dinucleotide is maintained through cell divisions which regulates the degree of transcription of the nearby genes and is important in developmental regulation of gene expression.

Cytoskeleton- A network of molecular filaments that includes actin, intermediate filaments, and microtubules that collectively act to provide structure for the cell.

DNA- Abb. Deoxyribonucleic acid. The molecules inside cells that carry genetic information and pass it from one generation to the next. DNA molecules are double-stranded. The two strands are complementary to one another and intertwine to form a double helix. Each strand of DNA consists of a chemically linked chain of nucleotides, each of which consists of a sugar, a phosphate and one of four kinds of aromatic bases - A, T, C and G. A and C residues on one strand pair, respectively, with T and G residues on the other strand. The ends of each strand of DNA are called the 5' and 3' ends. Enzymes read DNA strands in the 5' to 3' direction.

Dysmorphism- Abnormality of shape or configuration.

Exon- A coding sequence of DNA within a gene that is transcribed into RNA and that is subsequently translated into protein; "exons are interspersed with introns".

Farnesyl- A 15-carbon isoprenoid unit.

Farnesyl transferase- An enzyme that adds a farnesyl group to a cysteine amino acid at the C terminus of the Ras protein, a post-translational modification is required for Ras to become functional. Inhibition of this enzyme has become an attractive target for therapeutic intervention in tumours with aberrant Ras activation.

GABA- Gamma-aminobutyric acid, the major inhibitory neurotransmitter of the nervous system.

GAP- GTPase activating proteins (such as neurofibromin) which function to promote inactivation of small G-proteins (such as Ras) by stimulating its intrinsic GTPase activity, resulting in the hydrolysis of GTP to GDP and the release of a phosphate group.

Gene- An hereditary unit consisting of a sequence of DNA that occupies a specific location on a chromosome and determines a particular characteristic in an organism by directing the formation of a specific protein and is capable of replicating itself at each cell division.

Germ cells- Cells that will undergo meiosis to form the gametes (i.e., the ova and sperm).

Germline mutation- A mutation in germ cells that is passed on to offspring.

Haploinsufficiency- A situation where an individual, who is heterozygous for a certain gene mutation (often due to a deletion of the corresponding allele), is clinically affected because a single copy of the normal gene is incapable of providing sufficient protein production for normal function.

Heterozygous (+/-)- A situation in which a cell possesses two different alleles for the same gene; "+" indicates the wild-type allele; "-" indicates the variant allele. Sometimes the symbol "-" denotes a null allele, an allele that makes no gene product or whose product has no activity of any kine.

Homologous Chromosome- Two chromosomes, each derived from one parent, that are paired with one another, and contain the same linear gene sequences.

Homozygous (+/+, -/-)- A situation in which a cell possesses two identical alleles for the same gene.

Hyperphosphorylated tau- Covalent attachment of phosphate groups to the tau protein. This form of tau is thought to disrupt interaction with neuronal microtubules and is commonly found in patients with Alzheimer Disease (also see tau).

Intragenic- A situation in which a primary gene and the mutated gene lie within the same locus.

Intron- A non-coding sequence of DNA within a gene that is transcribed but is then subsequently removed by RNA splicing in the nucleus, leaving a mature messenger RNA consisting of only coding sequences that is then translated in the cytoplasm.

Iris hamartoma- Well defined dome shaped elevations projecting from the surface of the iris that appear yellow to brown and are a clinical feature of NF1.

Loss of heterozygosity (LOH)- A situation where heterozygosity for a deleterious mutant allele and a normal allele at a particular locus is lost due to a deletion or other mutational event within the normal allele, rendering the cell homozygous for the deleterious allele.

Long-term potentiation (LTP)- An in vitro, experimental model used to study synaptic plasticity mechanisms, events that underlie learning and memory.

Malignant- A tumour that is made up of cells that are no longer under normal growth control, and that have the potential to spread to and grow in another location in the body (metastasis).

Malignant peripheral nerve sheath tumour (MPNST)- Tumours that arise from peripheral nerve Schwann cells.

Meiosis- The process of cell division in sexually reproducing organisms that reduces the number of chromosomes in reproductive cells from diploid (having two sets of chromosomes) to haploid (having one set of chromosomes), leading to the production of gametes in animals and spores in plants. In meoisis I, two daughter cells that are diplopid are produced. In meiosis II, four daughter cells that are haploid are produced.

Mitogenic- An agent that promotes activation of signal transduction cascades resulting in mitosis.

Monogenic- Controlled by, or associated with a single gene.

Neoplasm- An abnormal new mass of tissue consisting of proliferating cells that serves no biological purpose.

Null (-/-)- A situation where both alleles of a particular gene are lost.

Neurofibrillary tangles- A structure composed of dense arrays of paired helical filaments (neurofilaments and microtubules) that are twisted into left-handed ribbon-like filaments. One of the hallmarks of the brain in patients with Alzheimer disease.

Neurofibromin- Protein product of the NF1 gene that functions to suppress Ras activation.

Oncogene- Mutated and/or overexpressed version of a normal gene of animal cells that in a dominant fashion can release the cell from normal restraints on growth, and that can convert a normal cell into a tumour cell.

Optic pathway glioma- Tumour that results from growth of abnormal cells in one or both optic nerves or the optic chiasm of the brain.

P53- A gene that normally regulates the cell cycle and protects the cell from damage to its genome. Mutations in this gene cause cells to develop cancerous abnormalities.

Paralogous- Two genes or clusters of genes at different chromosomal locations in the same organism that have structural similarities, indicating that they are derived from a common ancestral gene and have diverged from the parent copy by mutation and selection or drift.

Pilocytic astrocytoma- A subtype of astrocytoma (Grade I) that is biologically different from, and has better prognosis than, infiltrating astrocytomas (also see astrocytoma).

Plexiform neurofibroma- Tumours that arise under the skin. The word "plexus" refers to a combination of interlaced parts, or a network. Plexiform neurofibromas can grow in many places, such as on the face, down the leg or on the spinal column, and they can be disfiguring.

Point mutation- A mutation that causes the replacement of a single base pair with another pair.

Positional cloning- Identification of a gene based on its location in the genome. This usually results from linkage analysis based on a mutation in the target gene, followed by a chromosome walk from the nearest known sequence.

Ras- A member of the guanosine triphosphate (GTP)-binding proteins, that function transmit intracellular signals from cell surface receptors to the nucleus to regulate transcription of genes involved in cell growth and differentiation.

Signal transduction- Biochemical process in which a hormone or growth factor relays a signal from the cell exterior, through the cell membrane, and into the cytoplasm. These events involve a number of molecules, including receptors, proteins, and messengers.

Splice site- The site on an RNA molecule that is located between an exon and an intron at the 5' end of the intron. When the intron is removed during processing of heteronuclear RNA, the donor site is spliced to the acceptor junction at the 3' end of the intron.

Tau- A 60-70 kilodalton ubiquitously expressed protein that was the first microtubule associated protein to be characterized. Tau proteins, which are made by alternative splicing of a single gene, function to promote tubulin (the building blocks of microtubules) assembly. Dysregulated interaction of Tau with axon microtubules is thought to be a contributing factor in the pathogenesis of neurodegenerative diseases such as Alzheimer disease.

Theta-burst stimulation protocol- An electrophysiological protocol in which electrical impulses of varied duration, intensity and frequency are delivered to cultured brain slices in studies of long term potential (LTP) and long term depression (LTD).

Transcription factor- Proteins required for recognition by RNA polymerases of specific stimulatory sequences in eukaryotic genes. Several known transcription factors activate gene transcription by RNA polymerase II when bound to upstream promoters.

Tumour suppressor- Genes whose protein product function to restrain cell growth but when missing or inactivated by mutation, allow cells to grow in an uncontrolled manner.

Tumourigenesis- Process of tumour formation.

Untranslated region- Regions of a complementary DNA (cDNA), that are located 5' to the initiation (ATG) site or that are 3' to the stop site, which are not translated to make a peptide. The precise function of these regions are currently not well understood but are thought to be regulatory in nature.

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