The Neurofibromatosis Type 1 Syndrome and Associated Tumors

Steven L. Carroll, MD, PhD
Department of Pathology and Laboratory Medicine
Medical University of South Carolina

Disclosure of Relevant Financial Relationships

USCAP requires that all planners (Education Committee) in a position to influence or control the content of CME disclose any relevant financial relationship WITH COMMERCIAL INTERESTS which they or their spouse/partner have, or have had, within the past 12 months, which relates to the content of this educational activity and creates a conflict of interest. Dr. Carroll has nothing to disclose.

A 23 Year Old Man with Swelling of his Upper Right Arm

- A 23 year old white man presented complaining of fatigue and upper right arm pain and swelling that began several months earlier.
- He had no family history of neurofibromatosis type 1.
- On physical exam he was found to have several large café-au-lait macules and multiple dermal neurofibromas on his back.
- Neurologic exam showed no focal deficits in strength. Deep tendon reflexes were 2+ and symmetrical in the knees, ankles and hands.
- Multiple masses were palpable from the axilla to the antecubital fossa. These masses were very tender to the touch.

Guided by an initial biopsy, the patient underwent amputation of his right arm.

Pathology of the Mass

- Grossly, the mass had two distinct areas that merged into one another.
- The superior aspect (extending to the axilla) was yellow, foamy, tawny and distinct from adjacent tissues.
- The inferior aspect had a fish-flesh appearance, invaded adjacent tissue and extended subfascially along the humerus.
- In addition to immunohistochemical studies of the tumor, whole exome sequencing was performed on the tumor and peripheral leukocytes.

This showed a germline truncating mutation in the NF1 gene (p.R3253Stop).

Neurofibromatosis Type 1 (NF1)

- NF1 was first documented as an entity in the late 1800s, but descriptions of this disease can be traced to the 19th century.
- NF1 is the most common genetic disease affecting the human nervous system (1:1,000-3,000 newborns).
- Inheritance is autosomal dominant and affects males and females equally.
- Diagnosis requires at least 2 of the following:
  - Café-au-lait macules (V.5 cm postpubertal, V.8 cm prepubertal)
  - Axillary or inguinal freckling
  - One or more neurofibroma or two or more cutaneous neurofibromas
  - Optic glioma
  - Two or more 1.5 cm nodules
  - Bone dysplasia
  - Freckles that develop with NF1
- NF1 is incompletely penetrant. However, its manifestations are highly variable, even within the same pedigree.
Neurofibromatosis Type 1 (NF1)

- Non-neoplastic manifestations of NF1 include:
  - Pigmented lesions
  - café-au-lait macules
  - Axillary freckling
  - Soft, subcutaneous nodules (of the face)
  - Unidentified bright objects (UBOs) in basal ganglia, thalamus, brainstem, cerebellum or subcortical white matter (vascular malformations)
  - Round and exophytic subependymal nodules, overgrowth of abdominal aorta
  - Berry aneurysms
  - Macrophage
  - Short stature
  - Soft tissue, subcutaneous masses
  - Spheno-occipital dysplasia
  - Third ventricle
  - Learning disabilities

Testing for Germline NF1 Mutations

- The human NF1 gene, which is on the long arm of chromosome 17 (17q11.2), spans ~283,000 bp and contains 69 exons (some alternatively spliced) that encode a 2,814 amino acid (220 kDa) protein.
- Has one of the highest known de novo mutation rates (50% of cases are in families with no history of NF1); these mutations tend to be de novo during spermatogenesis.
- 95% of germline NF1 mutations are nonsense mutations, missense mutations, frameshift mutations or mutations affecting mRNA splicing. These can occur anywhere in the NF1 gene, but are most frequent in exons 5, 6, and 7.
- 5% of NF1 patients have whole gene deletions.
- Result from non-allelic homologous recombination (in Type 1, between NF1-REP 4 and NF1-REP 6; clusters of pathogenic deletions in Type 2 between FESFL and the FESFL enhancer).
- The best testing methods currently available identify NF1 mutations in ~90% of clinically suspicious cases. The remainder are mosaic/segmental NF1 or NF1 mimics (e.g., neurofibromatosis, von Recklinghausen Ueber die multiplen Fibrome der Haut und ihre Beziehung zu den multiplen Neuromen (1882).)

Does NF1 Genotype Predict Phenotype?

- Sometimes...
- NF1 microdeletion syndrome (the 5% of NF1 patients with whole gene deletion)
  - These patients have large numbers of dermal neurofibromas, dysraphic features and substantial cognitive impairment. They are at increased risk for development of plexiform neurofibromas and NF1-related malignancies.
- Patients with a 3 bp in-frame deletion in exon 17 of the NF1 gene lack neurofibromas but demonstrate the other manifestations of NF1.
- On the other hand, the manifestations of NF1 are highly variable, even within the same pedigree (and patients in the same pedigree carry the same NF1 mutation).
- This has led to the suggestion that modifier genes interact with NF1 to modify patient phenotypes. Possible modifier genes include NFI, NF2, NF3 and NF4.

Does NF1 Genotype Predict Phenotype?

- Sometimes...
- NF1 microdeletion syndrome (the 5% of NF1 patients with whole gene deletion)
  - These patients have large numbers of dermal neurofibromas, dysraphic features and substantial cognitive impairment. They are at increased risk for development of plexiform neurofibromas and NF1-related malignancies.
- Patients with a 3 bp in-frame deletion in exon 17 of the NF1 gene lack neurofibromas but demonstrate the other manifestations of NF1.
- On the other hand, the manifestations of NF1 are highly variable, even within the same pedigree (and patients in the same pedigree carry the same NF1 mutation).
- This has led to the suggestion that modifier genes interact with NF1 to modify patient phenotypes. Possible modifier genes include NFI, NF2, NF3 and NF4.

NF1 Patients Develop Several Types of Neurofibromas with Varying Malignant Potential and Distinct Natural Histories

- How neurofibromas are classified depends on who you’re talking to: pathologists, dermatologists, geneticists and basic scientists are all using different classification schemes at present. The scheme above is how pathologists do it in the WHO classification scheme.
- Although several of these neurofibromas subtypes have malignant potential, their malignant potential is variable (progression highest with malignant transformation of the others much less common).

Pathology of Neurofibromas

- Neurofibromas are composed of a complex mix of cell types
  - Schwann cells (the neoplastic component)
  - Fibroblasts
  - Epithelial-like cells
  - CD44-positive dendritic-like (T) cells
  - Mast cells
  - Macrophages
- Despite their difference in malignant potential, the histologic appearance of different neurofibromas subtypes is identical
- Other than NF1 LOH, genomic analyses show a normal diploid genome in neurofibromas

NF1 Loss in Schwann Cells and Hyperactive Paracrine Interactions Drive Neurofibroma Pathogenesis

- Loss of the remaining wild-type NF1 allele in a NF1– Schwann cell triggers deregulated proliferation and enhanced secretion of a variety of paracrine signaling molecules (e.g., Kit ligand).
- Other NF1 haplosufficient cell types in peripheral nerve are themselves hyperresponsive to the secreted factors and are recruited by the NF1 null Schwann cells to form the nascent neurofibroma.
Clinical and Pathologic Features of Malignant Peripheral Nerve Sheath Tumors (MPNSTs)

- MPNSTs are the most common malignancy (5-10% of neurofibromatosis cases) and the leading cause of death in NF1 patients (5-year cause-specific survival rate of 24.6%, compared to malignancies in general).
- MPNSTs account for 2-5% of soft tissue sarcomas and arise from the Schwann cell lineage. They occur in multiple settings:
  - 40-50% are unassociated with NF1, juvenile or familial NF (13 years)
  - 40% are associated with NF1, juvenile or familial NF (13 years)
  - (14.1%) arise as slips of previous lesions (mean latency 13 years)

  - Immunohistochemical markers have limited utility:
    - TP53: 50% or more positive
    - CDK4: 30-50% of cases positive
    - Loss of CDKN2A: Variable and depends on degree of Schwannoma differentiation

    - Basal-cell nevus syndrome markers (eg, PTEN, CDKN2A) are more frequent in low-grade MPNSTs
    - None. Can be difficult to determine one cell for the other

  - Uncommonly, multiple tumors can arise (importantly to poorly defined cutaneous lesions, with treatment removal, spontaneous regression may be more common in low-grade MPNSTs)

Unlike Neurofibromas, the Genomes of MPNSTs Are a Mess

- Classic karyotyping says MPNSTs are hypodiploid or near-triploid with multiple gains and losses
- High density SNP arrays with high-resolution lines and tumors show a characteristic pattern with unusually high levels of LOH and frequent gains
- LOH occurred in up to 20 chromosomes (median, 3; with gains to high copy number gains in up to 23 chromosomes (median 18)
- This suggests that there is a master list of genetic material only in MPNST pathogenesis followed by a whole or partial genome redistribution
- Whole-exome sequencing and exons sequencing also show common genetic rearrangements, resulting in the production of multiple fusion genes

Epigenetic Dysregulation Also Contributes to MPNST Pathogenesis

- Two groups performing whole exome sequencing of MPNSTs (Lee et al. Nat Genet 46: 1227-1232; Zhang et al. Nat Genet 46: 1179-1187) found that inactivating mutations of SUZ12 and EED, two components of Polycomb repressor complex 2 (PRC2), were present in up to 80% of MPNSTs
- SUZ12 and EED mutations are associated with a loss of h- and trimethylation of lysine 27 in histone 3 (H3K27me3 marks) and a transcriptional signature that is distinct from the signature of MPNSTs with intact SUZ12 and EED genes
- Restoring SUZ12 expression in SUZ12-null MPNSTs or restore H3K27me3 marks and inhibits tumor cell growth, indicating that this gene acts as a tumor suppressor in MPNST cells
- SUZ12 and EED are not lost in neurofibromas, indicating that SUZ12 and EED immunohistochemistry can be useful for distinguishing MPNSTs and neurofibromas

Targeting Ras-Dependent Pathways to Treat MPNSTs

- Since Ras is known to be difficult to target in MPNSTs, most efforts have focused on key downstream pathways (EGFR and mTOR)
- MEK inhibition has been effective in preclinical trials against neurofibromas but had limited effects against MPNSTs
- mTOR inhibition (targeting the PI3K-AKT-mTOR pathway) has had limited effects on Ras
- Combinatorial therapy with MEK and mTOR inhibition has a transient effect, but MPNST growth quickly rebounds

The Accumulation of Additional Genomic Abnormalities Drives Neurofibroma-MPNST Progression

- Initial studies identified several genes that were mutated in MPNSTs, but not neurofibromas:
  - TP53 mutations have been reported in up to 73% of MPNSTs (although other studies report that TP53 mutations are much less common)
  - TP53 inactivation is sufficient for MPNST pathogenesis
  - CDKN2A mutations, which deregulate the p53-Mdm2-p53 pathway, have similar effects. These mutations are found in 50% of MPNSTs
  - RAS activation has been observed in 20% of MPNSTs
  - PTEN loss, which deregulates the P13 kinase-Akt signaling pathway, is also evident in MPNSTs
  - EGFR and MTC amplification also occurs in MPNSTs

NFI Loss Results in Ras Hyperactivation

- Neurofibromin, the protein coded by the NFI gene, is a member of the GTPase activating protein (GAP) that inactivates Ras by stimulating GTP hydrolysis
- Concomitantly, activation of the remaining wild-type NFI allele in a NFI-/- Schwann cell results in hyperactivation of multiple classes of Ras (H-Ras, K-Ras, N-Ras, and S-Ras) family members
- It is evident how these NFI-/- Schwann cells override Ras-induced senescence
Targeting Receptor Tyrosine Kinases Upstream of Ras to Treat MPNSTs

Three weeks treatment

Genome Scale shRNA Screens Identify Signaling Pathways that are Vulnerable in MPNSTs

- Genome scale shRNA screens designed to identify genes essential for proliferation and/or survival were performed on 3 MPNST lines
- Genes that were "commonly essential" in a wide variety of cancer types (likely essential for basal metabolism) were excluded in each screen
- 107 genes were required in all 3 MPNST lines; pathway analyses identified two druggable pathways
- Agents targeting these pathways inhibited MPNST survival and proliferation

Tamoxifen and Trifluoperazine Inhibit MPNST Growth in Orthotopically Xenografted Mice

Acknowledgments

- Carrot Lab
  - Judy Lange
  - Amanda Prevel
  - Laurel Black
  - Brinna Jovy
  - Robert Wilson
  - Sue Wexler
  - Stephanie Breyer
  - Stephanie Britos
  - Nicola Ingenito
  - Jeff Eisel
  - Paul Pickford
  - Richard Povinelli
  - Richard Hajcak
  - Abhishek Choudhury

- NINDS
  - Katherine Hamming
  - Dina Wink

- Washington University School of Medicine
  - Robert Schrock
- Edna Alvis Institute for Neurotechnology
  - VNR Neuroinformatics Program
  - Interdisciplinary Neuroinformatics and Cognitive Neuroscience Core
- Comparative Health Translational
- Hollings Cancer Center

Sponsored by NINDS (R01 NS048353, F30 NS063626, F31 NS081824, T32 NS048039), NCI (R01 CA122804 and R01 CA134773), DO D (W81XWH-09-1-0086, W81XWH-11-1-0498 and W81XWH-12-1-0164), NIH (P30 NS057098), a UAB Comprehensive Cancer Center Pilot Grant, a UAB CTSA Pilot Grant and the Children’s Tumor Foundation