Neurofibromas
To Grow Or Not To Grow
That Is The Question

Michel Kliot MD
Clinical Professor
Department of NeuroSurgery
Co-Director of Peripheral Nerve Center
Stanford University School of Medicine
Stanford, CA
Once upon a time, patients presented with large, growing, and symptomatic tumors.
Therefore we have been biased to think that most tumors continue to grow

- These are the ones that were/are most likely to present clinically with symptoms and findings, and therefore often require a surgical intervention
Many tumors are being picked up incidentally at autopsy

- Prostate CA in 5% of men < 30 y/o to 59% of men > 79 y/o and 20% of men from 40 y/o to 70 y/o had high grade intraepithelial neoplasia without cancer
- Asymptomatic meningiomas in 1-2% of autopsies
- Incidental thyroid CA in 11.2% of autopsies
And...

- Many tumors are being picked up incidentally in screening studies.
- Natural history studies are showing that many tumors, particularly nerve tumors, either stop growing or grow very slowly.
- Randomized trials are being done comparing observation versus surgical treatment (Prostate Cancer) showing in some cases that the natural history of a disease may be better than a treatment intervention such as surgery.
Many nerve tumors stop growing for very long periods of time and therefore can be watched if asymptomatic.
Other Examples of Tumors That Stop Growing, Grow Very Slowly or Even Regress

- Acoustic Neuromas: ½ to 2/3 stop growing; work of Andrew Parsa showing variable growth
- Hemangiomas that regress
- High incidence of pituitary tumors that never come to clinical attention (15% incidence)
In epidemiological terms...

• As our sensitivity in detecting tumors has increased greatly...

• Our specificity in determining which tumors will become problematic has not kept pace
Snowball In A Blizzard
by Steven Hatch
Statistical Exercise

• NF 1 (1 in 3,500 mutation): about 100,000 people in US
• Assuming on average 10 nerve sheath tumors per patient = 1,000,000 NST’s
• We operate on maybe 10,000 per year
• 990,000 NST’s go untreated each year
To Grow Or Not To Grow
That Is the Question
A Big Challenge: The Natural History Of NSTs

• Variable and unpredictable tumor growth
  – Slowly growing NSTs
  – Rapidly growing NSTs (some of which are malignant and can metastasize)
  – Stable NSTs that stop growing for long periods of time
  – Regressing NSTs that actually get smaller
Variable Growth of Neurofibromas In A NF1 Patient
New Malignant Nerve Tumor in Right Medial Thigh In NF1 Patient
Many NSTs Stop Growing For Very Long Periods Of Time And Therefore Can Be Watched If Asymptomatic
Decision Tree: Follow With Serial Clinical Exams and MRI’s

- Incidental finding
- None or very tolerable symptoms
- Imaging evidence of no growth or very slow growth
Decision Tree: Operate

- Producing significant clinical symptoms and findings, especially if progressing
- Intolerable symptoms such as pain unresponsive to non-surgical treatments
- Imaging evidence of significant interval growth, particularly if in a dangerous location (e.g., spinal canal)
- Need a definitive diagnosis: percutaneous or open surgical biopsy
Peripheral Nerve Sheath Tumors: Surgeon’s Grading Scheme

Benign
- Surgically Cooperative
- Surgically UnCooperative

Malignant
Some NSTs Are Symptomatic, Benign, And Very Resectable Without Causing Functional Deficits
Right Distal Tibial Coop/Sticky NST
Intraoperative Ultrasound To Help Localize Masses
Right Distal Tibial Coop NST
R Prox Sciatic NST
(Ultrasound Is Our Stealth)
R Prox Sciatic NST (Cooperative) Using Mcevoy Butt Retractor
R Prox Sciatic NST (Cooperative)
R Prox Sciatic NST (Cooperative)
Some Benign NSTs Are Surgically A Little More Challenging To Resect
Some Benign NSTs Are Surgically A Little More Challenging To Resect
Left Post Cord Sticky NST: Removed Piecemal
Left Abdominal/Pelvic NF: Partially Resectable
Left Abdominal/Pelvic NF
R Radial NST W MR DTI
R Radial NST W MR DTI
R Radial NST W MR DTI
R Radial NST W MR DTI
R Radial NST W MR DTI
5 Months S/P Repair
Can We Use Imaging To Determine Resectability Of Nerve Sheath Tumors Preoperatively?
Right C4 Cooperative Schwannoma
MR DTI and Ultrasound
Malignant Left Sciatic Nerve Tumor

MRN DTI

Surgically Cooperative
L Buttock MPNT Adjacent To Sciatic N

GTR of Tumor

No Tumor W Sciatic Nerve Intact
Peripheral nerve sheath tumors offer us a very unique opportunity to answer these questions:

- By virtue of the fact that some patients contain both growing and non-growing tumors
- Allows us to look for molecular differences superimposed upon an identical genetic background
Huge Range in Phenotypic Expression of NF1 Genotype: From 0/1 to 100’s NFs
NF 1 Gene

• A very large gene on Chromosome 17
• Tumor suppressor gene that codes for the protein – neurofibromin – one of whose functions is it to regulate the RAS pathway which can influence cellular proliferation in a number of cell types including Schwann cells
NF1

- One of the most common genetic disorders (1 in 3,500)
- Displays an autosomal dominant pattern of inheritance
- Involves a germline or somatic cell mutation of at least one copy of NF1 gene
- Many different types of mutation have been found to involve the NF1 gene
- Some mutations are more common than others
- A second somatic cell mutation is necessary for phenotypic expression of NF1, involving either NF1 or another gene
Two Hit Hypothesis
Loss of Heterozygosity

- **normal chromosomes**
  - maternal, paternal

- **first hit**
  - mutation in tumor suppressor gene

- **second hit**
  - loss of wild-type chromosome

- **no functional tumor suppressor left**
Work In Progress

- Whole Exome Sequencing of Growing and Non-Growing Cutaneous Neurofibromas from a Single Patient with Neurofibromatosis Type 1

- Daniel L. Faden, Saurabh Asthana, Tarik Tihan, Joseph DeRisi, Michel Kliot

Whole Exome Sequencing Of Growing And Non-Growing Cutaneous Neurofibromas From A Single Patient with Neurofibromatosis Type 1

- Daniel L. Faden: Otolaryngologist affiliated with Upmc Presbyterian, Pittsburgh, PA
- Saurabh Asthana: Helen Diller Comprehensive Cancer Center, UCSF Medical Center
- Tarik Tihan: Department Of Pathology, UC SF Medical Center
- Jospeh DeRisi: Department of Biochemistry and Biophysics, Ucsf Medical Center
- Michel Kliot: Department of Neurosurgery, Stanford University School of Medicine
37 y/o woman with NF1 (2 of 7)
Clinical Criteria (at least 2 of 7)

• She had:
• Six or more café-au-lait spots or hyperpigmented macules (5 mm in diameter in prepubertal children and 15 mm postpubertal) – present at birth
• Two or more typical neurofibromas or one plexiform neurofibroma (she had resected as a child)

• She did not have:
• Axillary or inguinal freckles (>2 freckles)
• Two or more iris hamartomas (Lisch nodules), often identified only through slit-lamp examination by an ophthalmologist
• First-degree relative (eg, mother, father, sister, brother) with NF1
• Optic nerve glioma
• Sphenoid dysplasia or typical long-bone abnormalities such as pseudarthrosis
37 y/o woman with NF1

- Over a 1 year period of observation with several clinical exams of 14 symptomatic neurofibromas, based on patient’s report and my visual observations combined with crude skin surface measurements:
  - 11 showed no obvious evidence of growth
  - 3 showed evidence of growth

- Serial imaging was not done
Whole Exome Sequencing (WES)

- Sequencing only the DNA that codes for protein
- About 180,000 exons (30 million base pairs)
- About 1 % of entire genome (3 billion base pairs)
DNA Transcription

Transcription, elimination of intron transcript segments, and splicing of exons
Resection Of 14 Neurofibromas
In a NF1 Patient (3 growing and 11 non-growing)
Resection of 14 Neurofibromas
In a NF1 Patient (3 growing and 11 non-growing)
Resection Of 14 Neurofibromas
In a NF1 Patient (3 growing and 11 non-growing)
Resection of 14 Neurofibromas (+ 3 growing Neurofibromas)
Methods

• 6 neurofibromas sampled: 3 growing and 3 non-growing
• Typical neurofibroma features confirmed on neuropathological review of tissue specimens
• A blood sample obtained to reveal a germline mutation in NF1 gene
• Whole exome sequencing of 3 growing and 3 non-growing neurofibromas, as well as paired normal blood, was performed on an Illumina HiSeq 2000
Results

• Interrogation of the germline (blood) sample revealed a missense mutation in NF1
• Only 2 (1 growing and 1 non-growing) out of 6 neurofibromas had an identifiable somatic mutation involving NF1 gene
Results

- Between 1 and 11 mutations identified per neurofibroma sample
- Over-representation of mutations involving the HIPPO signaling pathway involved in regulating cell cycle progression, apoptosis, and cell differentiation
Results

• No obvious/consistent genetic differences between growing and non-growing neurofibromas
Table 1. Somatic Mutations in Growing and Non-growing Neurofibromas.

<table>
<thead>
<tr>
<th>Genes</th>
<th>Growing 1</th>
<th>Growing 2</th>
<th>Growing 3</th>
<th>Non-Growing 1</th>
<th>Non-Growing 2</th>
<th>Non-Growing 3</th>
<th>Variant Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>RASSF1</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>synonymous</td>
</tr>
<tr>
<td>TRAV9-1</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>missense</td>
</tr>
<tr>
<td>SUDS3</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>missense</td>
</tr>
<tr>
<td>KLHL20</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>frameshift</td>
</tr>
<tr>
<td>CD5</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>missense_variant</td>
</tr>
<tr>
<td>DLG4</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>missense_variant</td>
</tr>
<tr>
<td>WIBG</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>missense_variant</td>
</tr>
<tr>
<td>UNC5CL</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>missense_variant</td>
</tr>
<tr>
<td>HFE</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>missense_variant</td>
</tr>
<tr>
<td>HECW1</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>missense_variant</td>
</tr>
<tr>
<td>VPS36</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>synonymous</td>
</tr>
<tr>
<td>COL22A1</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>synonymous</td>
</tr>
<tr>
<td>UBASH3A</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>missense</td>
</tr>
<tr>
<td>CYFIP2</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>missense</td>
</tr>
<tr>
<td>PTPRZ1</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>sequence</td>
</tr>
<tr>
<td>RYR1</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>synonymous</td>
</tr>
<tr>
<td>NF1</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>stop gained</td>
</tr>
<tr>
<td>NF1</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>disruptive inframe deletion</td>
</tr>
<tr>
<td>DCAF13</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>missense</td>
</tr>
<tr>
<td>PSD2</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>missense</td>
</tr>
<tr>
<td>SLC25A14</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>synonymous, splice region</td>
</tr>
<tr>
<td>NIPBL</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>missense</td>
</tr>
<tr>
<td>ITIH1</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>missense</td>
</tr>
<tr>
<td>INA</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>missense</td>
</tr>
<tr>
<td>PRTG</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>missense</td>
</tr>
<tr>
<td>TTC28</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>missense</td>
</tr>
<tr>
<td>EIF4G1</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>missense, splice region</td>
</tr>
<tr>
<td>ABCC3</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>synonymous</td>
</tr>
<tr>
<td>SFN</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>synonymous</td>
</tr>
<tr>
<td>CYP17A1</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>missense</td>
</tr>
<tr>
<td>NOA1</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>missense</td>
</tr>
<tr>
<td>HLA-A</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>missense</td>
</tr>
</tbody>
</table>

https://doi.org/10.1371/journal.pone.0170348
http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0170348
Fig 1. HIPPO Signaling Pathway.

https://doi.org/10.1371/journal.pone.0170348
http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0170348
Conclusions

• Great deal of genetic heterogeneity among neurofibromas from same patient
• It is likely that both NF1 and Non-NF1 genetic mutations are playing important roles
• The senescent pathway genes we sampled did not distinguish between growing and non-growing neurofibromas
Activation of Senescent Pathways

• Senescence surveillance of pre-malignant hepatocytes limits liver cancer development

• Tae-Won Kang et al...

Conclusions

• Over-representation of mutations involving the HIPPO signaling pathway
• No obvious/consistent genetic differences between growing and non-growing neurofibromas in our pilot study
Caveats

- Very small n of 1 patient and 6 neurofibromas
- Need to more rigorously characterize the growth of neurofibromas over time with serial imaging measurements (MRI and perhaps ultrasound)
- Study many more NF1 patients
Conclusions

• Whether a neurofibroma continues to grow or stops growing likely involves a complex interaction between genetic and epigenetic factors both at a cellular and extracellular (environmental level)
Peripheral nerve sheath tumors offer us a unique opportunity to explore these molecular influences by employing:

- WGS/WES techniques
- mRNA chip technology
- Epigenetic modification (ChIP-seq) testing
- Proteomic analytic tools
Schwannomatosis

• Also known as NF3
• 1 in 40,000
• On chromosome 22 near NF2 gene
• Multiple Shwannomas including cutaneous
To Grow
Or
Not To Grow
That It Is
A Very Important Question!!
If we could understand why some nerve tumors grow while others do not

Instead Of Surgically Removing/Destroying Nerve Tumors (Car)

Put The Molecular Brakes On Nerve Tumors
If we had a way to predict the chances of whether a tumor would continue to grow or not, we could manage NF1 patients with greater confidence and efficacy.
Thank You!