Microtubule Defects in Neurological Diseases

Overview

Our understanding of neurological diseases has expanded rapidly in the last 20 years. If we looked back at 2002, neurodegenerative diseases like Parkinson's and Alzheimer's disease were treated as separate conditions. Psychiatric diseases like schizophrenia, bipolar disorder, and chronic, severe depression were thought to be more psychosocial conditions, not organic defects, and certainly separate from the neurodegenerative disorders.

Today the emerging model is that **many different neurological problems** can be traced (at least in part) to the same source: **microtubules**. We know neurons depend on microtubule networks in ways we did not understand before. When these networks fail, it leads to or accelerates neurological diseases. Which component(s) of a network have failed, and the cells of the nervous system in which the failure is most severe, determine which disease state occurs.

(This new perspective also could explain why many neurological diseases share similar clinical symptoms. For example, patients with advanced Parkinsonism, Alzheimer's, and white matter syndrome often experience similar bouts of visual and auditory hallucinations. )

Your goal for this week is to build a framework for understanding how microtubule defects lead to neurological disease.

* On Day 1 your team will use information you pull from two pre-class literature readings to work out how microtubules are arranged in differentiated cells, and more specifically, in neurons.
  + The larger learning goal is to practice pulling key concepts out of a noisy background.
  + One reading assignment (Muromoya 2017, pp. 1-6) has way more detailed information than you will need for the case. I recommend that you:
    - Read the guide questions your team needs to answer, then review the figures and read ONLY the first 1-2 paragraphs below each section or subsection heading.
    - See if you can answer some or all of the questions.
    - Only read further if you cannot answer a guide question.
* On Day 2 your team will use the general model you developed on Day 1 to understand what happens to neurons when microtubule-based activities break down.
  + The larger learning goal is to practice synthesizing a model using concepts from **more than one** source.
  + You will not be able to answer all of the Guide Questions for Day 1 or complete Day 2 using just the information in one of the reading assignments.
  + You will need to combine BOTH sources.

Your final assignment will be a summary of what you learned from the case.

Pre-Class Reading Assignment

**You do not have to read both articles.** Half of your team should read Sferra 2020, the others, Muromoya 2017. If you have an odd number, put the extra person on Muromoya 2017.

* [Muromoya 2017 up to p. 6](https://wakeforest.instructure.com/courses/44928/files/2865981?wrap=1)[Download Muromoya 2017 up to p. 6](https://wakeforest.instructure.com/courses/44928/files/2865981/download?download_frd=1), stop before “The stabilization and anchoring of microtubule minus ends”.
* [Sferra 2020 up to p. 10](https://wakeforest.instructure.com/courses/44928/files/2865980?wrap=1)[Download Sferra 2020 up to p. 10](https://wakeforest.instructure.com/courses/44928/files/2865980/download?download_frd=1), stop before “Section 5. Microtubule Dysfunction and Neurodegeneration.”

Both reviews contain information about vertebrates and invertebrates. For this case, you should focus on vertebrates only. The guide questions for the readings are in the next section.

Guide Questions for Day 1

### How are microtubules assembled and regulated?

Get with your team on Wednesday and complete these Guide Questions.

* 1. The proteins listed below are the main structural and regulatory proteins that make up the microtubule network. Using the readings, assign a general function to each of the proteins.
* CDK5RAP2
* CLASPs
* CLIPs
* Dynein
* HDAC6
* Katanin
* Kinesin
* MAP2
* MAPT/Tau
* Nedd1
* Ninein
* Sirtuin-2
* Spastin
* αTAT1
* α-tubulin (*TUBA#* genes)
* β-tubulin (*TUBB#* genes)
* γ-tubulin
* Tubulin tyrosine ligase (TTL)
* γ-TuRCs
  1. Which of the proteins in the list from Qu. 1 are **structural**, meaning they are part of the actual microtubule itself? Which of the proteins are **regulators**, meaning they control or modify stability, assembly/disassembly, etc.?
  2. What types of post-translational modifications do neuronal tubulins undergo? How do these modifications affect tubulin function?
  3. The basic model for microtubules is that the network radiates out from the centrosomes near the nucleus. This is not the only way they can be organized. How can the microtubule network be modified from the radial spokes model to serve differentiated cells? How does the microtubule change in differentiated neurons? Absorptive cells? Secretory cells? How does its arrangement reflect functions of each cell type?
  4. Why is finding a mechanism for nucleation in differentiated cells so important? What needs to happen in order for microtubules to anchor somewhere OTHER than the MTOC?
  5. In terms of regulation, what is the logic in having CDKs control where gamma-tubulin localizes in cells?

Day 2: How do microtubule network defects lead to neurological disease?

Your goal today is to use the core structure and regulation points you built last time to explain how microtubule defects lead to disease states.

Below I provided two lists of options to choose from. Each team gets to choose one (and no, not everyone can do Alzheimer’s!) I recommend picking from the Primary Options, but if you feel daring choose one of the less well-studied diseases in the Alternate Options.

For the disease that you select, use outside sources to supplement your understanding and develop answers to these questions:

1. What are the clinical diagnostic features? What are the cellular diagnostic features?
2. Based on the model you developed for Day 1, how does or could the cellular features explain the clinical features?
3. Where is the main defective protein normally? Where is it located during the disease state?
4. How is it possible for several different neurological diseases to arise from the same general defect (i.e., microtubule dysfunction)?

### Primary Options

These are well-established as having associated microtubule defects.

* Schizophrenia
* Progressive Supranuclear Palsy (PSP)
* Primary age-related tauopathy (PART) dementia
* Prion diseases (Crutzfeldt-Jakob disease, chronic wasting disease)
* Parkinson’s disease (PD)
* Hereditary Spastic Paraplegia (HSP)
* Frontotemporal dementia (FTD)
* Amyotrophic Lateral Sclerosis (ALS)
* Alzheimer’s disease (AD)

### Alternate Options

These have less evidence, conflicting data, or both.

* Bipolar disorder
* Chronic traumatic encephalopathy (CTE)
* Corticobasal degeneration (CBD)
* HDAC6 over-activity
* Ischemic stroke
* Lead encephalopathy
* Sirtuin 2 defect
* Tubulin tyrosine ligase deficiency

### What You Will Hand In

1. Your table or illustration summarizing the functions of the main microtubule proteins from Day 1. Make sure to include whether each protein acts mainly as a structural component, or a regulatory component.
2. A summary description of the disease that you selected. It should include:

* What are the clinical diagnostic features? What are the cellular diagnostic features?
* Based on the model you developed for Day 1, how does or could the cellular features explain the clinical features?
* Where is the main defective protein normally? Where is it located during the disease state?

1. A short answer to this question: how is it possible for several difference neurological diseases to have similar causes?

Case and Instructor Notes:

This case comes at the end of a junior-level cell and molecular biology course. It is the capstone for several cases in which students have practiced extracting and interpreting information from increasingly complex datasets and primary literature reading assignments. To complete this case students work in teams to assemble a general understanding of how microtubules are organized and function in neurons. Students evaluate the quality and accuracy of their framework by using it to describe the role(s) of microtubule defects in one neurological disease.

The case intentionally is very open ended. Students have spent considerable time developing their skills over the semester. This is their opportunity to test those skills prior to going into the final exams. Many of the students enrolled in this course are on a pre-health track, so this also provides the opportunity to apply course concepts to disease conditions.

### Case Day 1 Questions:

* 1. **The proteins listed below are the main structural and regulatory proteins that make up the microtubule network. Using the readings, assign a general function to each of the proteins.**
  2. **Which of the proteins in the list from Qu. 1 are structural, meaning they are part of the actual microtubule itself? Which of the proteins are regulators, meaning they control or modify stability, assembly/disassembly, etc.?**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Proteins** | **Structural** | **Mixed** | **Regulatory** | **Function** |
| CDK5RAP2 |  |  | x | Cdk5 regulator. Ties m’tubule stability to cell cycle. |
| CLASPs |  | x |  | +-end protein. Stabilizer |
| CLIPs |  |  |  | + end protin. Stabilizer |
| Dynein | x |  |  | Motor protein for travel to - end |
| HDAC6 |  |  |  | Cytoplasmic histone deacetylase |
| Katanin |  | x |  | Severs microtubules |
| Kinesin | x |  |  | Motor protein for travel to + end |
| MAP2 |  | x |  | Lateral regulator of stability |
| MAPT/Tau |  | x |  | Lateral regulator of stability |
| Nedd1 |  |  | x | Targets γ-tubulin to organizer locations |
| Ninein |  |  |  | - end anchor protein for epithelium |
| Sirtuin-2 |  |  |  | NAD-dependent deacetylase |
| Spastin |  | x |  | Severs microtubules |
| αTAT1 |  |  | x | A primary tubulin lysine acetylase |
| α-tubulin (TUBA# genes) | x |  |  | First of 2 proteins in heterodimer |
| β-tubulin (TUBB# genes) | x |  |  | Second of 2 proteins in heterodimer |
| γ-tubulin | x |  |  | Basal – end anchor in MTOCs. Regulates CLIP binding |
| Tubulin tyrosine ligase (TTL) |  |  | x | Addition half of -Y/+Y cycle on tubulin. |
| γ-TuRCs |  | x |  | Gamma tubulin ring complex. |
|  |  |  |  |  |

* 1. **What types of post-translational modifications do neuronal tubulins undergo? How do these modifications affect tubulin function?**

Tubulins can undergo acetylation and de-acetylation, cyclic tyrosine removal and addition, and glutamylation. Some of these modifications change microtubule stability, either accelerating or blocking catastrophic depolymerization. There is a large amount of evidence to say that these modifications also act as a regulatory coding system, similar to how epigenetic modifications of histones regulate DNA activity.

Many microtubule-related proteins have preferential affinities for microtubules (or domains of a microtubule) that are enriched or deficient in certain modified tubulins. For example, kinesin-1 prefers microtubules rich in detyrosinated and acetylated tubulins, while kinesins 5 and 13 prefer microtubules rich in tyrosinated tubulins. Other proteins sensitive to the tubulin code include microtubule-severing proteins and some + end polymerases.

The tubulin code is regulated by highly conserved and tightly regulated enzymes, which might only be found in selected areas of cells. For example, the enzymes may be differentially enriched or regulated in axons versus dendrites, enabling axonal microtubules to be richer in post-translationally modified tubulin subunits compared to dendritic microtubules.

* 1. **The basic model for microtubules is that the network radiates out from the centrosomes near the nucleus. This is not the only way they can be organized. How can the microtubule network be modified from the radial spokes model to serve differentiated cells? How does the microtubule change in differentiated neurons? Absorptive cells? Secretory cells? How does its arrangement reflect functions of each cell type?**

In differentiated neurons, the microtubules are largely arranged in bundles parallel to the primary axon. This provides a path for moving neurotransmitter vesicles and other organelles to and from the synapses. Absorptive cells often have microtubules with the “-“ end anchored to or near the plasma membrane to assist with endocytic vesicle transport. Secretory cells have an increased density of microtubules connecting the MTOC to the apical membrane region, again to support rapid transport prior to export.

Collectively these differences show how microtubule arrangement is matched to the main direction of intracellular transport in differentiated cells.

* 1. **Why is finding a mechanism for nucleation in differentiated cells so important? What needs to happen in order for microtubules to anchor somewhere OTHER than the MTOC?**

Cells must have a way to create additional nucleation points if they are to undergo complex trafficking. Otherwise cells cannot change the classical radial organization seen in dividing cells, and vesicles could only go a few places inside of cells.

* 1. **In terms of regulation, what is the logic in having CDKs control where gamma-tubulin localizes in cells?**

Adding the cdk control ties microtubule stability and structure to cell cycle. Cells cannot form the differentiated organization patterns as long as a cell is getting pro-growth signals.

### Case Day 2 Questions:

* 1. **What are the clinical diagnostic features if the disease you chose? What are the cellular diagnostic features?**
  2. **Based on the model you developed for Day 1, how does or could the cellular features explain the clinical features?**
  3. **Where is the main defective protein normally? Where is it located during the disease state?**

**4. How is it possible for several different neurological diseases to arise from the same general defect (i.e., microtubule dysfunction)?**

These are the same questions that students must turn in at the end of this case. Summary responses are below.

##### What Students Should Turn In

* 1. **Your table summarizing the functions of the main microtubule proteins from Day 1, and whether each protein acts mainly as a structural component, or a regulatory component.**

Students’ tables should resemble the one in the key for Day 1.

* 1. **A summary description of the disease that you selected. What are the clinical diagnostic features? What are the cellular diagnostic features? Based on the model you developed for Day 1, how does or could the cellular features explain the clinical features? Where is the main defective protein normally? Where is it located during the disease state?**

The table below lists microtubule defects linked to tubulin mutations.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Locus | Change | Location | Model | Effects | Source |
| TUBB3 | R262H, R262C | Intermediate | Mouse | Defects in axon guidance | Tischfield et al., 2010 |
| TUBB2B | E421K | C-terminal | Mouse | Axonal dysinnervation | Cederquist et al., 2012 |
| TUBB2B | S172P | N-terminal | Rat | Impaired microtubule assembly, defective migration of cortical neurons | Jaglin et al., 2009 |
| TUBB2B | F265L | Intermediate | Rat | Impaired microtubule assembly, defective migration of cortical neurons | Jaglin et al., 2009 |
| TUBB2A | N247K | Intermediate |  | Cortical dysplasia | Cushion et al., 2014 |
| TUBB3 | T178M, E205K | N-terminal |  | Malformation of cortical development and neuronal migration defect | Poirier et al., 2010 |
| TUBB3 | A302V, M323V | Intermediate |  | Malformation of cortical development and neuronal migration defect | Poirier et al., 2010 |
| TUBB4A | D249N | Intermediate |  | Leukodystrophy | Simons et al., 2013 |
| TUBB4A | R2Q, T178R | N-terminal |  | Leukodystrophy | Miyatake et al., 2014 |
| TUBB4A | R53Q | N-terminal |  | Hypomyelinating leuko-encephalopathies | Miyatake et al., 2014 |
| TUBA4A | W407X | C-terminal |  | ALS | Smith et al., 2014 |
| TUBB4A | R2G, R53G | N-terminal |  | DYT4 dystonia dysphonia | Lohmann et al., 2013 |

See the outlines below for the data students should be able to summarize.

**Alzheimer’s disease (AD)**

In Alzheimer's disease, abnormal chemical changes cause tau to detach from microtubules and stick to other tau molecules, forming threads that eventually join to form tangles inside neurons. These tangles block the neuron's transport system, which harms the synaptic communication between neurons.

**Amyotrophic Lateral Sclerosis (ALS)**

**Bipolar disorder**

**Chronic traumatic encephalopathy (CTE)**

**Corticobasal degeneration (CBD)**

**Frontotemporal dementia (FTD)**

Loss of nuclear-cytoplasmic transport due to tau accumulation in cell body, leading to track errors.

**HDAC6 over-activity**

**Hereditary Spastic Paraplegia (HSP)**

Genetic mutations in the microtubule-severing enzyme Spastin, is most commonly associated with hereditary spastic paraplegia (HSP) (Hazan et al., 1999). Spastin loss of function results in local accumulation of detyrosinated microtubules (Tarrade et al.,2006) and reduced number of dynamic plus-ends marked by EB3 along the axon shaft (Fassier et al.,2013). These mutations also result in axonal swellings which can be rescued by treatment with microtubule-destabilizing drugs such as Nocodazole (Fassier et al.,2013). This hyper-stability of microtubules likely triggers the progressive degeneration of corticospinal tracts in the Spastin-dependent HSP cases(Hazan et al., 1999; Evans et al.,2005). Collectively these studies demonstrate that reduced dynamics of microtubules is also detrimental to neuronal health.

**Ischemic stroke**

**Lead encephalopathy**

**Parkinson’s disease (PD)**

**Primary age-related tauopathy (PART) dementia**

There are more than 80 Ser/Thr phosphorylation sites and 5 potential tyrosine phosphorylation sites in human tau. The biological activity of tau is regulated by the degree of its phosphorylation. Low phosphorylation level of tau in normal brain is required for its interaction with tubulin, in order to promote its assembly into microtubules and help stabilize their structure.

Some kinases and phosphatases have been implicated in hyperphosphorylation of tau. The main aberrantly hyperphosphorylated sites on tau include the phospho-sites Ser202/Thr205, Thr-231/Ser-235, Ser-262, Ser-396/404 and Ser422.11-14 Phosphorylation of Ser202/ Thr205, Thr-231/Ser-235 and Ser-262 significantly decreases the interaction of tau with microtubules. Phosphorylation of Ser-396/404 increases the propensity of tau to oligomerize and eventually form filamentous aggregates.

Cyclin dependent kinase 5 (CDK5) and glycogen synthase kinase 3β (GSK3β) have been identified as major candidates mediating tau phosphorylation at disease-associated sites. Ser-396/404 is a major target of GSK3β, while Ser202/Thr205, Thr-231/Ser-235 and Ser-262 are major targets for CDK5. CDK5 is an atypical cyclin-dependent kinase and its activity is dependent upon interaction with p35/p39.

**Prion diseases (Crutzfeldt-Jakob disease, chronic wasting disease)**

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Expression of GSK3β in the brains of scrapie-infected animals is downregulated and its target p-tau (Ser396/Ser404) is depressed as well. In contrast, the activities of both CDK5 and GSK3β are demonstrated to be increased, which are involved to regulate tau hyperphosphorylation in Alzheimer disease.

The different pattern of two kinases between AD and prion disease may lead to different pathological effects of tau. In prion disease, the predominant phosphorylation of tau are those that decrease the ability of tau to bind microtubules by CDK5 rather than those increase the ability of tau to self-associate and aggregation by GSK3β. That might explain why it is hard to observe NFTs in most types of prion diseases, except in some GSS cases.

**Progressive Supranuclear Palsy (PSP)**

**Schizophrenia**

Tyrosination/detyrosination of a-tubulin occurs in cycles. Detyrosination confers stability whereas tyrosination increases microtubule dynamics. Catalyzed by tubulin tyrosine ligase (TTL), tyrosination is important in neurons during axonal growth and transport [Magiera and Janke, 2014]. Detyrosination refers to the removal of Tyr from the C-terminal of a-tubulin to expose a Glu residue. Glutamylation takes place when the side chains of glutamates are added to the carboxy terminal tails of a– and b-tubulin by enzymes belonging to the tubulin tyrosine ligase-like (TTLL) family. This occurs at high levels in the nervous system. Deglutamylation is catalyzed by cytosolic carboxypeptidase enzymes, which are capable of removing glutamate side chains. Polyglutamylation changes the surface charge of tubulin, and influences the binding of MAPs and motor proteins [Janke, 2014].

Impaired protein transport and disrupted binding of MAPs is proposed to contribute to the underlying pathology in schizophrenia [Ikegami et al., 2007]. The TTLL11 polyglutamylase is linked to schizophrenia where a combined disruption in TTLL11 (a balanced t(9;17) (q33.2;q25.3) translocation, and a microduplication at 16p13.1) is associated with an additive predisposing effect [Fullston et al., 2011]. Interestingly, this same chromosomal aberration is also linked to bipolar disorder [Rajkumar et al., 2014].

Polyglycylation of a- and b-tubulin is analogous to polyglutamylation except that of glycines are added instead of glutamates. This modification is limited to cilia and flagella. The responsible enzymes are again members of the TTLL family [Fukushima et al., 2009]. Tubulin glycylases and glutamylases are important for stabilization and motility of ependymal cilia, which line the ventricles of the brain [Grau et al., 2013], while interestingly, neuropsychiatric risk genes have been shown to converge on regions encoding proteins found in cilia [Marley and von Zastrow, 2012].

**Sirtuin 2 defect**

Acetylation/deacetylation of a-tubulin K40 occurs within the microtubule lumen; catalyzed by acetyltransferases aTAT and Atat-2, whereas b-tubulin can be acetylated on K252 by the Sun acetyltransferase leading to a block of free tubulin assembly [Magiera and Janke, 2014]. Deacetylation, on the other hand, is executed by the histone deacetylase 6 (HDAC6) and nicotine adenine dinucleotide-dependent deacetylase sirtuin-2 (SIRT2) [Hubbert et al., 2002]. The anti-depressant action of HDAC inhibitors in a variety of rodent tests for depression has been recently reviewed [Fuchikami et al., 2015]. Although most HDACs act on histones in the nuclear compartment, HDAC6 is cytosolic and acts on tubulin.

**Tubulin tyrosine ligase deficiency**

* 1. **A short answer to this question: how is it possible for several difference neurological diseases to share similar causes?**

Disease patterns can be result of:

* Microtubule insufficiency overall
* Microtubule misalignment, mis-orientation (for example, + ends point wrong way)
* Structural defects (g-tubulin or anchor defects, CLASP errors, etc.)
* Errors in MAP1b, MAP2, Tau regulatory proteins

The particular location within neurons, the subpopulation affected, and when in the cellular life cycle it fails can all change the disease phenotype or cells affected.