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CourseSource

Lesson

Understanding the Importance of Microtubules in Normal Cell Division: Chemotherapy Connection

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Abstract

In introductory courses, students learn about microtubules as important structures but may not engage in a hands-on experience to localize microtubules themselves or to learn about their connection to cancer treatment. In this lesson, students review microtubule structure and function and then design a concept map based on what they have learned. Students also conduct an immunofluorescence procedure using budding yeast cells to observe microtubule localization at different stages of cell division. This technique involves using alpha-tubulin-specific antibodies which work on both yeast and mammalian cells. In the second part of the lesson, students examine their results from the immunofluorescence procedure using fluorescence microscopy and begin to explore different classes of chemotherapy drugs that alter microtubule structure in eukaryotic cells. They also search a clinical trials database to find examples where these microtubule-altering drugs are used for cancer treatment. Many students may have heard of chemotherapy as part of a first line treatment for cancer but may not understand how certain drugs disrupt microtubules to stop cancer. Students report back what they have learned about the different classes of microtubule drugs in small groups, and then add to their concept maps to introduce where a drug may alter microtubule structure and/or function. Using a combination of on-line tools and in class laboratory work, this lesson strengthens students' understanding of microtubule structure and function, critical to the life of the cell. Students are assessed for their understanding of the topic in several ways, including as a part of a laboratory exam.

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Learning Goals

Students will:

◊ Part I
- » recognize microtubule structure and know the critical role of microtubules in cell division.
- » understand microtubule dynamics in live cells.
- » conduct the technique of immunofluorescence and understand the use of antibodies to detect microtubules.

◊ Part II
- » know how to analyze cells that have gone through the immunofluorescence procedure.
- » identify mitotic spindles and other structures like spindle pole bodies at different stages of the cell cycle.
- » comprehend how microtubules contribute to the uncontrolled growth seen in cancer.

◊ From the Biochemistry and Molecular Biology Learning Framework:
- » How are structure and function related?

◊ From the Cell Biology Learning Framework:
- » How do the different components of the cytoskeleton support a variety of cell functions, such as cell shape, division, movement, sensing the environment, and cell-cell communication?
- » How do cells conduct, coordinate, and regulate nuclear and cell division?
- » How do the methods and tools of cell biology enable and limit our understanding of the cell?

Learning Objectives

Students will be able to:

◊ perform an immunofluorescence experiment to localize microtubules in cells, including the proper controls.

◊ define and organize microtubule functional information relative to structural details using a concept map.

◊ explore different classes of microtubule disrupting chemotherapy drugs.

◊ investigate how the chemotherapy drugs are currently being used alone or in conjunction with other drugs to treat cancer using clinicaltrials.gov information.

◊ present their findings on the different types of chemotherapy drugs to the class and use this information to modify their original concept map.

Learning Goals

◊ From the Genetics Learning Framework:

- » How do the results of molecular genetic studies in model organisms help us understand aspects of human genetics and genetic diseases?
- » What experimental methods are commonly used to analyze gene structure and gene expression?

INTRODUCTION

Microtubules are an important part of the eukaryotic cytoskeleton, participating in cell motility as a part of cilia and flagella, and in cell division by making up the spindle fibers that attach to kinetochores to move chromosomes, among other roles. Each microtubule is a polymer assembled from alpha-tubulin and beta-tubulin subunits, linked to make a filament, with filaments joining together to make a hollow tube displaying dynamic instability (1, 2). This lesson involves the use of budding yeast as a model organism, a unicellular eukaryote which is known for being cheap, easy to grow, and easy to manipulate to make genetic mutants (3, 4). Budding yeast microtubules are well conserved, being remarkably like microtubules found in other eukaryotes. The budding yeast mitotic spindle is also like the mammalian spindle, although mitosis is "closed," with the spindle forming inside the nuclear envelope (5–9).

Students learn the basics of microtubule structure and function in introductory biology courses, with a focus on how eukaryotic cells use microtubules to move chromosomes during mitosis. However, in a more advanced cell biology or molecular biology course, one can begin to discuss how the use of live cell microscopy techniques, like fluorescence microscopy, revealed the importance of the dynamic instability and treadmilling of microtubules for their function (2). Microtubules are dynamic in the sense that they are individually being built up and broken down continually when not stabilized by binding to another structure, and they can move in a treadmill-like motion in a particular direction by adding subunits to one end as subunits are removed from the other end. Microtubule treadmilling and dynamic instability can happen at the same time, and microtubule movements increase dramatically in mitosis relative to interphase. These characteristics make microtubules more vulnerable to the action of chemotherapy drugs targeting microtubules, during this time (10, 11).

Although microtubule structure and function are routinely covered in lecture-style courses, this topic is not typically the focus of a laboratory or hands-on exercise. However, a few resources are available that can be used to enhance classroom instruction. Textbooks often have short videos available as supplementary information to demonstrate microtubule dynamics, and I have used these resources to complement lectures and prompt in-class discussion (2). Video reviews, such as those from the *Journal of Cell Biology*, curate some of the best videos on microtubule movements found in the literature (12). Several education articles have been published about studying microtubules and their role in cell division in a virtual lab or an in-person lab setting (13, 14). In addition, there are articles describing lessons on the role of microtubules in meiosis and in development using models (15, 16). A laboratory exercise has also been published to study the role of the microtubules and actin filaments in contraction of cultured rat heart cells (17). I present here a lesson which complements these other useful resources, taking the original approach of conducting immunofluorescence to visualize microtubules in yeast, combined with concept map design to look at the role of microtubules in mitosis and the effect of microtubule-altering drugs.

Microtubule destabilizing agents affect the ability of the alpha and beta subunits to build and maintain a microtubule polymer. Alternatively, stabilizing agents prevent the breakdown of the polymer locking the microtubule in a state when it cannot be dynamic (10, 11, 18–20). The first microtubule stabilizing agent discovered, paclitaxel (Taxol), is a natural product which comes from the bark of a Yew tree (18, 21–23). (My students took notice of the name of this tree because of its importance in the Harry Potter books!) Taxol is also famous for being a part of a program to screen natural plant products for their anticancer activity (22). If Taxol is used to stabilize microtubules, it leads to a phenomenon known as mitotic catastrophe where cancer cells form multipolar spindles that attempt to complete mitosis but leave the daughter cells with abnormal numbers of chromosomes (22, 24). Cellular resistance to cancer drugs is a big issue, and this lesson also covers chemotherapy drug resistance, and how cancer cells can use several methods to become less susceptible to these drugs (19, 25, 26). In the second part of the lesson, as students are studying different microtubule-altering drugs, they consult the Clinicaltrials.gov database, which catalogues clinical studies that can be searched for any cancer-related study involving a particular microtubule-altering drug (27).

This lesson was created during the COVID-19 pandemic, when I modified the original immunofluorescence in-person laboratory lesson planned for my Cell Biology of Cancer course so that it could be completed through remote learning. To improve the lesson and keep some of the original learning goals, I decided to dive into microtubule structure and the functional connection between blocking microtubules and treating cancer. I added student activities such as designing concept maps and small group work with on-line resources on cancer drugs. A remote learning version of this lesson was presented at a "Catalyst Conversation" education conference during the pandemic (28).

Before starting the lab, I review several key concepts using a handout on microtubule structure and function (Supporting File S1). The handout can be discussed either at the start of the first laboratory session or the last lecture class before the lab. I review the function of microtubules in eukaryotic cells, with a focus on cell division. An overview of microtubule structure

is also discussed, including the construction of a microtubule from the subunits to the polymer, in addition to the GTP energy requirement. Finally, microtubule dynamics in live cells are detailed. This material is based on content originally presented in an introductory cell biology course required of all biology majors, a prerequisite for my Cell Biology of Cancer course. In addition, we explore the ways to study microtubule structure using microscopy, to help put the immunofluorescence procedure that they would conduct in a broader context (2). I also provide an example from my own collaborative work on using electron tomography to visualize cellular structures like microtubules (29).

In the first of two laboratory sessions on the lesson (2.5 hours), students execute the steps of an indirect immunofluorescence procedure (IF), see Supporting File S2. This technique is based on the method outlined and modified over many years by yeast geneticists for a Cold Spring Harbor Laboratory course (30, 31). This technique is called indirect immunofluorescence because an unlabeled primary antibody is applied to the sample, in this case an antibody against alpha-tubulin, and then a secondary antibody linked to the fluorescent dye, FITC, is applied the next day to bind to the primary antibody. The microtubule localization can then be visualized using fluorescence microscopy (2). By assigning different concentrations of primary antibody to different student groups, students participate in the same type of protocol optimization that would be conducted by researchers in the field. In addition, one can also vary what kind of sample is given to students, wild type or a yeast mutant, to demonstrate how a well-known technique can still be relevant when asking new questions related to understanding microtubule structure.

During the lesson, students also create a concept map and revise it based on added information during the laboratory. The process of creating a concept map and using it for course assessment has been described elsewhere (32, 33). Concept maps can be a useful tool to evaluate the understanding of key concepts and how they are connected to one another, in this case, how to build a microtubule and how this structure relates to its dynamics and function.

In the second of the two full laboratory sections for the lesson (2.5 hours), students examine their immunofluorescence results by working with me, one group at a time, to image their slides using our fluorescence microscopy system (Supporting File S3). This is typically done when the students are sitting with me at the research microscope taking the images. Points of discussion on the IF experiment include the comparison of experimental to control samples, and how varying the dilution of the primary antibody altered the outcome.

Students complete Part Two by giving informal group presentations of their findings on their assigned drug. As a part of the worksheet assignment, students are asked to revisit their original concept maps from Part One to now add the chemotherapy drugs into the map. I had shown the students samples of their fellow students' concept maps at the beginning of the period, so they had the option to modify their entire map design based on what appealed to them from a fellow student's map.

Hopefully, students will come away from this lesson with a better understanding of microtubules, how to localize them in the cell and their importance in controlling cell division which can be exploited to fight cancer. This lesson fits into the context of other laboratory lessons in several ways. Before this lesson, one could conduct an on-line laboratory session to review cell division and the stages of mitosis, focusing on the role of microtubules, using microscopic images available on-line or through other sources (13). Another approach is to provide more context for budding yeast as a model research organism. There is a *CourseSource* lesson available on the life cycle of budding yeast and the effect of its environment on cell division (34). A six-part lesson, studying the effects of microtubule destabilizing or stabilizing drugs on mammalian cells, can be used to accompany or extend this lesson. This lesson involves fluorescently labeling the cytoskeleton in mammalian cells (14). Finally, besides lab-based exercises, the *CourseSource* lesson on analyzing experimental colorectal cancer data to understand the steps of tumorigenesis has also been a useful group exercise in my cell biology of cancer course (35).

Intended Audience

The intended audience for this lesson includes junior and senior level biology, biochemistry and molecular biology majors who have taken at least one cell or molecular biology course. Most recently, this lesson was used in an upper level four credit Cell Biology of Cancer lecture and laboratory course populated by biology majors.

Required Learning Time

This lesson typically takes two 2.5-hour laboratory periods, with an additional 1.5 hours required between the two laboratory periods. This additional time is required to complete the immunofluorescence experiment started in the first laboratory period, since a consecutive day is needed to do the secondary antibody incubation. I typically use time normally devoted to lectures for this step.

Prerequisite Student Knowledge

Students should come to the lesson familiar with introductory level information including the definition of the cytoskeleton as provided in an introductory biology course. Since the immunofluorescence procedure used for microtubule localization involves antibodies, familiarity with antibodies as a tool to study proteins would also be important. If this lesson is used for a course other than Cell Biology of Cancer, students should be familiar with the uncontrolled cell growth seen in cancer, since they will learn how microtubules contribute to this uncontrolled growth.

Prerequisite Teacher Knowledge

Instructors should be familiar with the background knowledge listed in the student knowledge section above. Familiarity with the cellular changes in cancer addressed by chemotherapy would be important. Familiarity with designing concept maps and using on-line databases would be relevant. Finally, if the instructor chooses to conduct the immunofluorescence experiment, familiarity with culturing budding yeast cells using conventional microbiology technique would be helpful in addition to experience with doing an antibody-based procedure like immunofluorescence.

SCIENTIFIC TEACHING THEMES

Active Learning

Active learning is a critical part of effective scientific teaching (36). This lesson uses active learning techniques in several ways. In parts one and two, students actively engage in concept map development to organize and express their understanding of microtubule structure and function. The students then adapt the concept map to latest information about that they learned about the anti-cancer drugs. Concept maps not only involve students linking key terms using arrows, but also deciding what relationships to represent between the terms overall (37). Concept maps also appeal to a student's sense of creativity, especially since students have many options for how they want to create the maps (33). In addition, concept maps can help the students retain the information more readily than just memorizing definitions (37). In part two, students engage in group work and then discussion to learn about distinct kinds of anti-cancer drugs. Students are guided with a series of questions, but then they decide how they want to present the information orally. This assignment also strengthens student communication skills.

In this lesson, students also perform an immunofluorescence experimental protocol on their own, not just look at the finished product of someone else's experiment to examine microtubules. Students learn about and encounter potential pitfalls in the procedure, observe the value of controls, and see how outcomes may vary from expected results. If a yeast mutant is employed for the experiment, students can see first-hand how microtubules might be altered in that sample.

Assessment

After completing part one, students receive feedback on their concept maps and are shown examples of different concept map representations by their fellow students before part two. At that point, students will modify their maps to add the chemotherapy drugs into the picture, or redesign their maps, if they choose to do so, based on ideas that they get from other students' concept maps. In part two, student work is assessed by the successful presentation of the group's results from the chemotherapy drug study. Students also complete worksheets for attendance for laboratory parts one and two. The lesson principles and major results are covered on the final lab exam containing multiple choice and short answer type questions (Supporting File S4). Overall understanding of the purpose of both laboratories is assessed by the summary paragraph narratives written by the students for their laboratory portfolio/notebook.

Inclusive Teaching

Using a variety of in-class and out-of-class assignments and group work, this lesson is meant to be accessible to all different kinds of students, and to be conducted keeping in mind the principles of an inclusive classroom, including creating a supportive classroom environment (36, 38). The lab groups for this lesson are typically assigned by the instructor, formed in a way that students are working in new groups for different projects throughout the semester, to encourage diversity in the student group makeup (39). Typically, the enrollment in an upper-level course is approximately sixteen students with four working groups at a time. There is some evidence that concept mapping can be a valuable assessment tool for determining student expert knowledge in science, independent of GPA and economic resources available to the student (40).

LESSON PLAN

Initial Instructor Preparation (see Table 1)

Potential yeast strain details and yeast growth media protocols can be found in another *CourseSource* lesson and other places (34, 41, 42). A wild type *Saccharomyces cerevisiae* strain (S288C background) can also be purchased from the American Type Culture Collection (43). The yeast strain should be maintained on an agar plate that can be used to start a liquid culture. The instructor should be familiar with sterile technique to avoid bacterial contamination (44). Also, the instructor will need to grow up an overnight yeast culture to obtain logarithmic-phase cells that can be fixed and saved in the refrigerator a few days before part one of the laboratory (30, 31). I also pre-treat the cells with the buffers required in the initial steps of the protocol, so that students can go right into the zymolyase treatment of the cells in the first laboratory session. Students can participate in any of the pre-lab preparations if the lesson is to be extended over more lab periods.

The instructor should purchase diagnostic immunofluorescence slides with a special Teflon template added to create wells for different samples on the slide, typically ten wells. In addition, the components of the buffers and solutions for the IF procedure, the poly-lysine solution to coat the wells, and the zymolyase to digest the yeast cell wall so they can take up the antibodies should be obtained in advance (30, 31). Other than these supplies, standard lab pipettors, pipette tips, microfuge tubes and a plastic box for a slide incubation chamber should be used. A commercially available rat monoclonal antibody against tubulin, YOL1/34, was used for this study and was a well-known, reliable primary antibody for IF (45). The secondary antibody was a Donkey anti-Rat IgG, FITC conjugate from the same company, although an antibody tagged with a different fluorescent dye would work too.

Part One: Lab Week 1 Immunofluorescence Procedure (2.5 Hours)

Pre-Lab

Students are given the laboratory handouts in advance, including the worksheet (Supporting File S2). They should review the PowerPoint slides (Supporting File S1) ahead of time. In the handout, students are asked to answer questions on two short videos related to microtubule dynamics. These videos include: Eukaryotic Cell Division and Self-Organization in Biology: Microtubules.

Lab

To start the laboratory, I review the background material on microtubules and microtubule dynamics. Then, we go over the first part of the IF procedure. Students are assigned to four groups of four, and each group is given a different fixed yeast sample in a microfuge tube. In brief, students pre-treat slide wells with the poly-lysine solution, zymolyase treat the cells, and then add the cells to the wells to settle in the wells. Cells are then treated with chemical to help fix them to the slides, washed with buffer, and the overnight primary antibody incubation is started in the incubation chambers created using plastic boxes which are maintained at room temperature in a place where they will not be moved around before the next class meeting (30, 31).

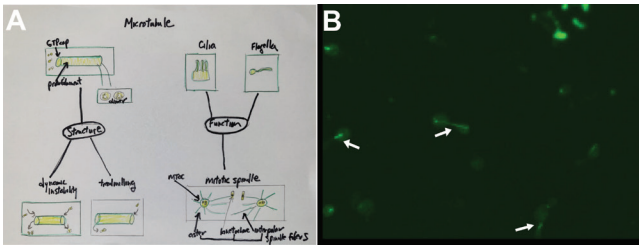

Figure 1. Concept map and immunofluorescence results. Shown here is an example of a concept map **(A)** of the structure and function of microtubules. The map was created by the author based on the best examples of student work. In **(B)** is a fluorescence microscopy image of typical results of the student immunofluorescence procedure using budding yeast. The microtubules are indicated with white arrows.

Before the lab is adjourned, I introduce the idea of creating a concept map and provide a link with sample instructions and examples. Even though the students work in groups for the IF procedure, they complete individual concept maps and worksheets (see Figure 1A).

Students leave the first laboratory period with the assignment to design their own concept map using a word cloud that I created as a source of key terms for the map. Students implemented a few approaches to make the maps, including drawing a map freehand and sending me a picture, using a word processing program to create a chart or table-type concept map or employing commercially available websites with templates (46, 47).

Concept map creation and discussion can be conducted in a completely remote learning environment. I reviewed each student's concept map before the second laboratory period to assess student knowledge of the topic, suggest any necessary changes and find good examples of different approaches to share with the other students.

Post-Lab

Students are asked to submit worksheets, including their concept map, for lab attendance. Worksheets not only include questions on the pre-lab videos, but also on the IF procedure itself, for example, summarizing major steps and identifying proper controls.

Since IF is a two-day procedure, one for primary antibody incubation and one shorter period for secondary antibody incubation, students finished the experiment during lecture class the next day (1.25 hour), and then I preserved the samples on the slides using an anti-fading agent and stored the slides in the refrigerator so the cells can be observed during the next laboratory session.

Part Two: Lecture Class Meeting for Secondary Antibody Incubation (1.25 Hour)

The next day during the lecture period, students remove their slides from the incubation chambers and perform a series of washes to remove the primary antibody. Then, the secondary antibody is added and incubated for approximately one hour. During this time, I typically conduct a normal lecture class for the duration. Cells are washed, the last buffer added, and the students give their slides to the instructor. I apply an anti-fade agent underneath a glass coverslip for each slide and store the slides in the refrigerator (protected from the light) until the next laboratory period.

Part Three: Lab Week 2 Immunofluorescence Analysis and Chemotherapy Drug Investigation On-Line (2.5 Hours)

Instructor Prep

The instructor will just need to remove the slides from the refrigerator about 30 minutes before the class so that students can start imaging their cells at the start of lab.

Pre-Lab

Students are given the part two laboratory handout in advance including a worksheet. They are asked to watch two videos: a video on mitotic catastrophe, which can be induced by chemotherapeutic drugs, and a brief video defining chemotherapy. In the first video, mitotic catastrophe is caused by an apoptosis-inducing protein. Students are asked to describe the stages that they see in the video in their worksheet questions, including what happens to the microtubules. The second video begins by focusing on the historical origins of chemotherapy.

Lab

At the start of lab, the instructor should review the key parts of the lab handout, including the chemotherapy drugs. In addition, the instructor can present examples of individual concept maps (with names removed) from the class and ask the students to discuss some of the key points that they like in each map. Students perform two main tasks during today's meeting. First, they are asked to image their IF slides, with the help of the instructor, using the fluorescence microscopy system (Leica DM5500, Buffalo Grove, IL). A conventional epifluorescence microscope is sufficient to observe the slides, a confocal microscope is not needed (see Figure 1B). Second, students work in small groups, ideally different than their IF experimental groups, to conduct group work on different classes of microtubule-altering drugs. Students are asked to revise their original concept map to include the drug from their group. Once they research certain properties of these drugs, including how they bind to microtubules, then they go to the Clinicaltrials.gov website to find examples of these drugs currently being used in clinical research on cancer. Drug assignments include agents such as estramustine, vinblastine, docetaxel and vincristine. A general overview of the drugs with links to resources for the students can be found in the part two lab handout. After all the students have looked at their IF experimental results and completed their group work, the student groups take turns presenting their findings to the rest of the class.

Post-Lab: Worksheet and Concept Map

With the information that they know now after the group presentations, students are asked to add all the drugs to their original concept map and upload it with today's worksheet. Students also could completely revise their original concept map in case they like another style or approach better than their own.

TEACHING DISCUSSION

Summary

During this lesson, students learned about the structure and function of microtubules, microtubule dynamics in cells and how certain chemotherapy drugs disrupted microtubules to cause mitotic catastrophe. Students explored in depth the

types of drugs affecting microtubules in different ways, and how those drugs are used for clinical cancer research. Concept mapping is used twice during the lesson to assess student knowledge of the initial topic and how the drugs interfere with microtubules. By performing an immunofluorescence procedure, students learned how to visualize microtubule structure and determine if it was disrupted.

Observations of Effectiveness, Overall Student Reactions Class Performance

A remote learning version of this lesson was conducted in 2020, and the full lesson including immunofluorescence was performed in 2022. Students were able to successfully complete the lesson in both cases. Students were assessed on their understanding of the topic through formative assessment involving worksheet questions, concept maps, a group presentation, and through summative assessment including a lab exam and lab portfolio entries summarizing the lesson.

In the Cell Biology of Cancer course, the laboratory grade is 40% of the 1000 points in the course. Completion of the worksheet and successful creation of their original concept map, along with the updated map, were a part of the 25-point attendance grade for the course. Their performance on a laboratory exam covering this lesson and others was worth 50 points. Their summary paragraph on the laboratory sessions for this lesson was completed as a part of their final laboratory portfolio/notebook for the course worth 50 points. Images of their IF results are included in the lab data section of the portfolio/notebook. Sample lab examination questions can be found in Supporting File S4.

The students liked working in teams since they were able to help each other learn the material, especially when discussing the chemotherapy drugs and reviewing the concept maps. They found that explaining something to someone else was one of the best ways to learn something well. The students enjoyed making the concept maps and most had never made a concept map before for another class.

Possible Improvements, Adaptations or Extensions for Different Courses or Student Populations

This particular lesson can be used in several kinds of courses including a molecular biology or biochemistry laboratory course. Since the immunofluorescence experiment involves the use of antibodies, there are many opportunities to expand the lesson to talk about the five kinds of human immunoglobulins (antibodies), monoclonal versus polyclonal antibodies, and the many other applications like ELISA using antibodies (2, 48). In my Cell Biology of Cancer course, this laboratory complements lecture content on deregulation of the cell cycle and on cancer treatments.

If this lab were expanded to be a half-semester or semester long, one could repeat the immunofluorescence experiment on drug-treated samples (such as adding the microtubule depolymerizing drug nocodazole) or use an unknown sample for the students, in an approach like longer laboratory lessons published with *CourseSource* (14). Students could design their own hypotheses and experiments using one of a number of yeast mutant options such as a yeast mutant sensitive to Taxol, or a cold-sensitive tubulin mutant known as *tub2-401* (49, 50). Using microtubule-altering drugs on the yeast strains would connect the on-line component researching certain chemotherapy drugs directly to the procedure conducted in the laboratory. Students could also image the microtubules using a different technique besides immunofluorescence, such as using a yeast strain where tubulin is tagged with CFP or GFP to follow the microtubule dynamics in live cells.

If one preferred to conduct this laboratory in a virtual learning environment, most aspects of the laboratory exercise could still be used, such as the review of microtubule structure and function, the microtubule movies, the concept maps, the research on chemotherapy drugs using Clinicaltrials.gov. In the past, in place of the immunofluorescence procedure, I have showed students images from past experiments conducted in previous years, and asked students to interpret the images. One could also add comparisons to microtubules from other model organisms, such as mammalian cells, using on-line resources such as the Chroma Image Gallery or the "Images as Phenomena" resource of the HHMI BioInteractive webpage (51, 52).

There are also ways to extend the lesson if one is not conducting the in-person experimental component, including expanding the concept map portion of the lesson. One could make the concept maps collaborative, even having the students assemble the map together using a group document on-line or on the board in the classroom (32, 33). In addition, although I ask the students to create their initial concept map using the terms in a word cloud, one can ask the students to add their own terms to expand the map, making it more detailed as they progress through the lesson (33, 37). If one has even more time to devote to the concept maps, one can ask the students to derive the concept maps completely from scratch, adding in a brainstorming session of key terms for the map first, a stage often used with this practice (32). To assist with grading, there are also rubrics or assessment guidelines available if one wants to grade the concept map itself (33, 37).

SUPPORTING MATERIALS

- S1. Chemotherapy Connection – Microtubule structure and function review PowerPoint
- S2. Chemotherapy Connection – Lab 1 handout including worksheet
- S3. Chemotherapy Connection – Lab 2 handout including worksheet
- S4. Chemotherapy Connection – Sample laboratory exam questions on the lesson

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Table 1. Procedure for understanding the importance of microtubules in normal cell division: chemotherapy connection. This table illustrates the main steps of the immunofluorescence preparation and procedure, as well as other activities associated with the two-lab period, one-lecture period lesson.

Activity	Description	Estimated Time	Notes
Preparation for Lab Module			
Making yeast media	• YPD agar to maintain yeast strain • YPD media (100 ml) to grow overnight yeast culture before starting preparation of cells for IF	2 hours	• See (34)
Putting yeast strain on agar plate	• Needed a few days before week 1	20 minutes	• A stock of yeast strain can be stored in -80 °C freezer in YPD plus glycerol • Yeast strain can be grown up on agar plate at room temperature for several days, then maintained in refrigerator on plate up to 3 weeks
Making buffers and solutions needed for Immunofluorescence (IF)	• Needed for week 1	3 hours	• See (30, 31)
Student pre-lab work	• Students review microtubule PowerPoint and watch movies	45 minutes	
Week 1: Immunofluorescence (IF) Procedure			
Background Information	• Instructor reviews pre-lab work and goes over IF procedure	30 minutes	• Supporting File S2
Students conduct IF procedure in small groups	• Students fix cells to slide, perform washes and primary antibody incubation	2 hours	• Also see (30, 31)
Week 1: Secondary Antibody Incubation During Lecture Period			
Students finish IF procedure	• Students wash slides, do secondary antibody incubation (1 hour waiting period) and conduct last buffer application	1.25 hours	• Also see (30, 31)
Preserving slides until week 2	• Instructor adds anti-fade agent and coverslips, stores slides in refrigerator protected from light	30 minutes	
Week 2: Observing if Results and Group Work on Microtubule-Altering Drugs			
Discussion and background information	• The class discusses their concept maps • Instructor reviews new background information and movies • Students discuss their interpretation of mitotic catastrophe movie	30 minutes	• Supporting File S3
Observation of IF slides	• Students meet with instructor in microscope room in small groups • Discussion of experimental and control samples	1 hour	
Group work on chemotherapy	• Students work in small groups to research the chemotherapy drug assigned to their group, including role in clinical research • Students modify concept maps based on their findings	30 minutes	
Class discussion	• Groups take turns reporting out findings about chemotherapy drugs • Students not presenting take notes related to how to modify their concept maps to reflect all drugs discussed	30 minutes	

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