Phalloidin has helped elucidate how cancer kills … and how it responds to radiation treatments and chemotherapy.

A Poison Becomes a Means to Cure

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Humankind has a knack for using poisons to its advantage. From ancient hunters lacing their arrow tips with paralyzing toxins to modern dermatologists smoothing skin wrinkles with the agent of botulism, humans excel at turning deadly substances into useful tools. This is especially true in the biochemistry laboratory where the physiologic manipulations caused by toxins can be repurposed to further our understanding of how diseases work and how to more effectively treat them.

Kingdom Fungi is one important natural source of toxins, a prime example being phalloidin found in the mushroom *Amanita phalloides*. Also known as the "death cap," *A. phalloides* is one of the deadliest mushrooms on the planet. It counts among its victims emperors, caesars, and tsars through the centuries. Ingesting sufficient quantities of the death cap mushroom can lead to acute, fulminant liver failure resulting in yellowing of the skin (jaundice), swelling of the brain, and hemorrhage. For such patients, urgent liver transplantation is the only cure.

The death cap's legendary toxicity, which is partly due to phalloidin, has become an asset to biologists studying the inner workings of human cells. And it is helpful for the same reason that it is toxic – phalloidin's affinity for a protein called "actin."

Actin is a strong fibrous protein that was originally found in the body's skeletal muscles where it facilitates contraction of muscle fibers. Scientists later found actin to be an important part of the fibrous skeleton inside every human cell. This "cytoskeleton" allows cells to maintain their shape and move, and, for cancer cells, it allows them to invade surrounding tissues and metastasize to distant locations in the body.

The role of actin in the cytoskeleton depends on its unique ability to lengthen and shorten. While each actin molecule exists as a globular protein, the individual globules stack up like Legos, or polymerize, to form tiny filaments. The proteins can then separate again, or depolymerize, to shorten. Actin globules inside a cell are continually polymerizing and depolymerizing to make movement and other processes happen, a process of rapid turnover known as "treadmilling" which is demonstrated very well in this video: https://www.youtube.com/watch?v=VVgXDW_8O4U.

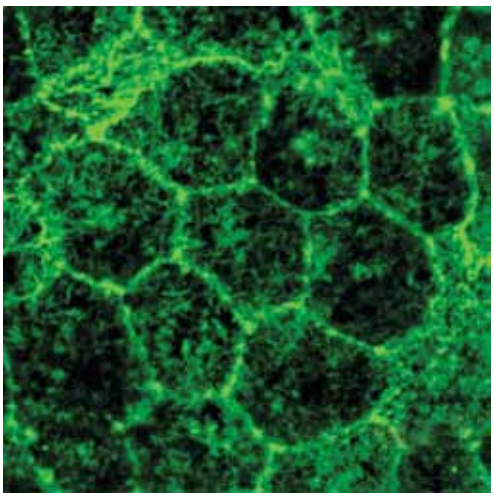

Fig. 1. Phalloidin illuminates in green the actin fibers of the cytoskeleton, including where actin connects each cell to its neighboring cells.

All photos courtesy of Christine Chiasson-MacKenzie.

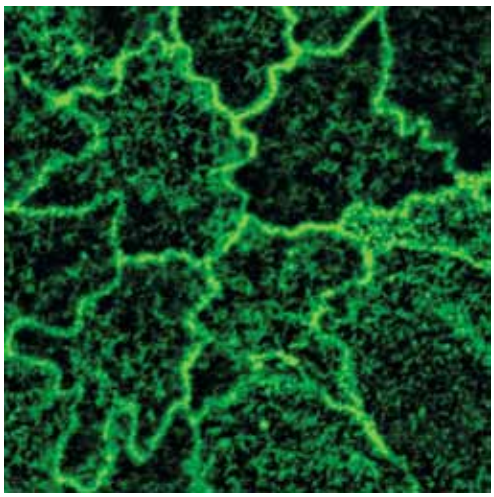

Fig. 2. Phalloidin staining demonstrates the altered cytoskeleton and jagged cell borders in the disease neurofibromatosis type 2.

Phalloidin kills human cells by disrupting actin's ability to polymerize and depolymerize. The toxin binds strongly to the filaments made up of polymerized actin, locking the protein and preventing it from depolymerizing into individual actin globules again. Essential cell functions depend on both actin's lengthening and its shortening, and so phalloidin causes disruption of the cell membrane, impaired cell function and, ultimately, cell death. Though phalloidin has the ability to poison any cell in the body by this mechanism, since all cells have cytoskeletons, it impacts the liver most because that organ plays a dominant role in the body's management of ingested toxins and readily takes up phalloidin from the bloodstream. Though actin is plentiful inside of muscle cells, the toxin is not taken up as much by muscle cells and therefore does not significantly impact muscle function.

Phalloidin's affinity for actin creates useful opportunities to turn a poison into a research tool. Before administering phalloidin to cells under study, a flourescent dye is bound to the toxin. Once inside the cells, the toxin homes toward and binds tightly to actin, as it does in mushroom poisonings, but in this case it brings the fluorescent dye in tow. The cell's cytoskeleton becomes decorated with flourescence, and shining a light with the correct wavelength onto the cells illuminates the actin, allowing scientists to clearly visualize the cytoskeleton. The resulting vibrant images are captured with special cameras.

This technique has led to new understandings of cell

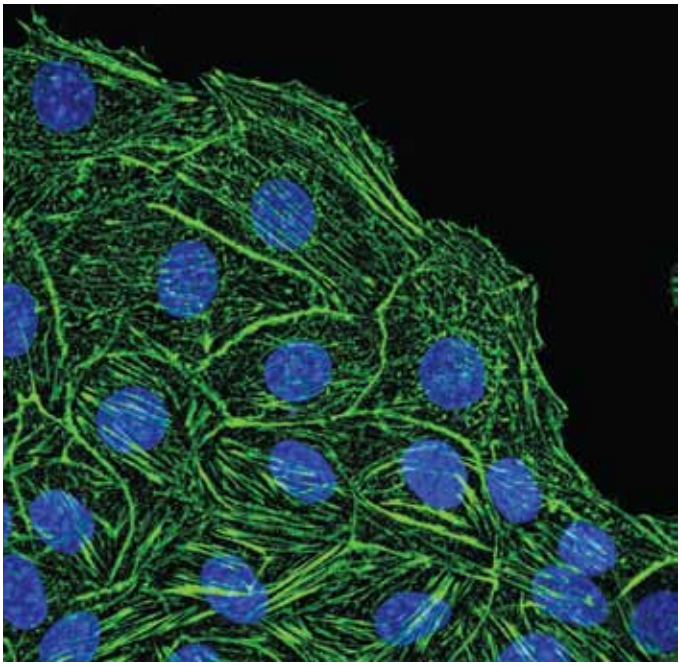

Fig. 3 Phalloidin illuminates in green the cytoskeleton of mouse liver cells. Cell nuclei are stained blue with a different technique that does not use phalloidin.

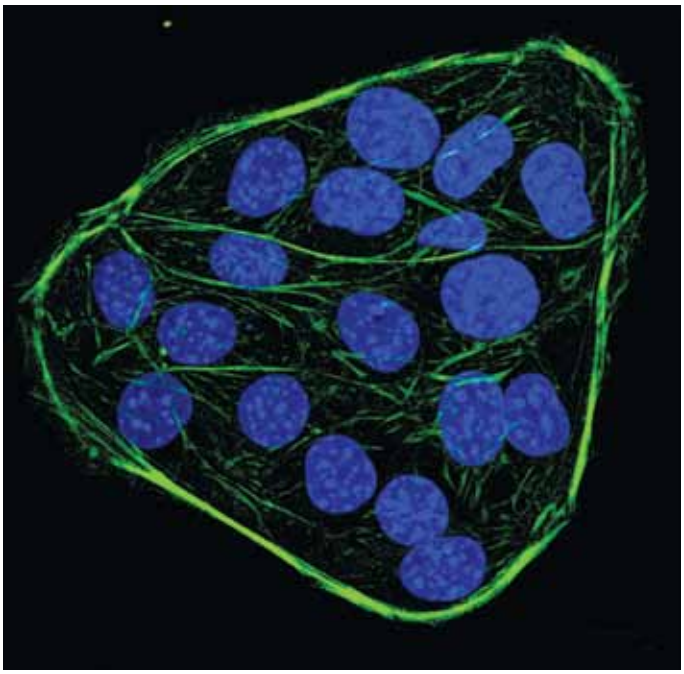

Fig. 4. Another view of mouse liver cells stained similarly to Figure 3.

function, and phalloidin has become a standard stain used widely in cell research. It has helped elucidate how cancer kills and how it responds, or fails to respond, to radiation treatments and chemotherapy. Professor Andrea McClatchey at Massachusetts General Hospital's Cancer Center regularly uses phalloidin to study how cancer cells move and migrate, and how they shape themselves during cell division. While she laments the 60-plus pages of documentation needed to purchase the deadly mycotoxin for use in her lab, she is hopeful that this toxin, which has killed many humans throughout history, can help give life back to many more by leading to better cancer treatments. When asked about the dangers of working directly with phalloidin, Professor McClatchey reported no cases of phalloidin poisoning in her lab, since very low concentrations of the toxin are used in staining cells. "We are not nearly as careful with phalloidin as we are with some other more dangerous lab toxins" she says.