Optochemistry to control the microtubule cytoskeleton

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Microtubule drugs have a wide range of applications in cell biology research as well as cancer therapy; however their application was so far limited to the treatment of entire cell populations and tissues. In a recent paper in *Cell*, Borowiak *et al* (2015) now describe a novel type of switchable microtubule drugs. The activity of their drugs, denoted as “photostatins”, can be switched on and off by violet and green light, respectively, which allows for the first time a precise spatial and temporal control of the microtubule cytoskeleton in single cells and tissues.

See also: M Borowiak *et al* (July 2015)

Microtubules are dynamic filaments and, as such, key components of the eukaryotic cytoskeleton. They are ubiquitously present in all known cell types and fulfill a wide range of essential functions in, for example, cell division, cell morphology and polarity, cell motility, and intracellular transport. During cell division, microtubules establish the mitotic spindle, a highly complex structure in charge of the faithful segregation of the chromosomes into the two daughter cells. The fascinating precision by which the intrinsic dynamicity of microtubules is coordinated with the activity of different microtubule-associated proteins and molecular motors to build the mitotic spindle has driven many key discoveries in the microtubule field. Moreover, analysis of the basic mechanistic principles underlying spindle assembly and function has provided key insights into the origins of aneuploidy, which is the most pertinent hallmark of cancer (Holland & Cleveland, 2012).

A strict control of their dynamics is essential for microtubules to adapt to different functions inside cells. At the same time, altering microtubule behavior is exploited in the treatment of diseases, in particular cancer (Dumontet & Jordan, 2010). Small ligands that interfere with microtubule dynamics, referred to as microtubule-targeting drugs, have been extensively used in basic research as well as in chemotherapy, the most famous example being Taxol. By binding tubulin molecules, which are the building blocks of microtubules, these drugs either destabilize or stabilize microtubules, thus interfering with their dynamics, and consequently with their physiological functions.

Microtubule drugs are commonly used to study the role of the cytoskeleton in living cells. For example nocodazole, a drug that reversibly depolymerizes microtubules, is frequently used in experiments to demonstrate the dependence of cellular processes on the microtubule cytoskeleton. Notably, the molecular mechanisms of action of several microtubule drugs on tubulin and microtubules have been recently elucidated at medium to high resolution by X-ray crystallography (Ravelli *et al*, 2004; Prota *et al*, 2013) and cryo-electron microscopy (Alushin *et al*, 2014).

In cancer chemotherapy, the systemic application of microtubule drugs provokes a number of detrimental side effects, such as cardio- or neuro-toxicities (Milteneburg & Boogerd, 2014). It would therefore be highly desirable to have in hand drugs with a much higher selectivity to specific microtubule subtypes that are, for example, over-expressed in tumor cells, and/or drugs that can be spatially and temporally controlled in a precise manner.

Photostatins are small molecules based on the microtubule-destabilizing drug combretastatin A-4 that binds to the colchicine site between the α - and β -subunit of the α/β -tubulin heterodimer (Fig 1A). Borowiak and co-workers have changed the chemistry of combretastatin by substituting its bridging C=C double bond with an isosteric N=N double bond. This modification allows the reversible switching of the drug from an almost completely inactive *trans* conformation into a highly active *cis* conformation using visible light (Fig 1B). While the drug turns into an active, sub-micromolar cytotoxin after illumination with violet light, it is more than 200 times less toxic in the dark. Green light in turn can be used to re-convert the photostatins into their inactive form (Fig 1B).

The authors went on describing how the dual color activation/deactivation mechanism enables a wide spectrum of new opportunities for cell biology research. They show how microtubules can be selectively depolymerized in single cells incubated with a photostatin, simply by shining focused light on one cell in a cell culture dish. The activated drug efficiently disassembled the entire microtubule cytoskeleton of the illuminated cell in less than a second, while none of the surrounding cells were affected. The authors further showed that the drug also functions in an intact tissue: Using *Caenorhabditis elegans* embryos incubated with photostatin, they were able to switch the drug into its active state specifically in only some selected cells. These cells were promptly arrested in cell division, while neighboring cells continued dividing. Finally, the authors tested the efficiency of photostatins in mammalian tissues. The

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Figure 1. Colchicine-binding site in tubulin and switching of photostatin.

(A) Crystal structure of the complex formed between α/β -tubulin (ribbon representation) and colchicine (sphere representation) at 2.4 Å resolution (Prota *et al.*, 2014). (B) Optical *cis*–*trans* switching of photostatin (PST-1). Only the active *cis* isomer is thought to bind to the colchicine site of tubulin (Borowiak *et al.*, 2015).[#]

mouse cremaster muscle was incubated with the drug *in vivo*, and then single cells were illuminated with the activating violet light in the live tissue. Again, microtubules in the illuminated, but not in the neighboring cells, were efficiently depolymerized.

The development of photoswitchable microtubule drugs is a large technological leap forward in both, fundamental biology and pharmacology of the microtubule cytoskeleton. Being able to reversibly modulate microtubules in selected cells, or subpopulations of cells *in vivo*, opens many exciting opportunities to study microtubule-related cytoskeletal functions in different

type of cells, as well as in entire organisms. Similar to the powerful approaches of optogenetics, which have in the past years become important tools for cell biology research (Miesenbock, 2011), “optochemistry” opens up new methodological opportunities in cytoskeleton research. Moreover, approaches using photostatins can profit from the optical instrumentation that has already been developed for the use of optogenetics in cells and in particular in whole animals. While the use of optochemistry in cultured cells and small, transparent model organisms such as zebrafish or *Caenorhabditis elegans* will be rather straight-forward,

more technological developments will be needed to apply similar tools on optically less accessible regions of larger organisms like mice. In the meantime, it seems conceivable that the depolymerization of microtubules in sub-regions of cells will be possible using photostatins: To achieve a spatially restricted effect, only a small window of the cell could be illuminated with the activating violet light, while the rest of the cell is protected by the inactivating green light. In this way, diffusing drug molecules in the activated state that enter the protected regions of the cell will rapidly be transformed into their inactive form, and vice versa.

The potential medical applications of photostatins are also exciting, although several additional developments will be necessary to make these drugs suitable for the clinic. The light activation in the visible regime is perhaps the biggest problem in the use of these drugs in cancer therapy, as penetration of visible light is highly limited by the depth of the targeted tissue. The development of similar drugs that could be activated, for example, in the infrared regime, might be able to partially circumvent this problem, as infrared radiation can easier penetrate into thicker tissues. Another way of locally using these drugs is to combine them with surgery, which would allow direct illumination of the operated tissue. Apart from cancer treatment, other applications of microtubule drugs have recently been demonstrated, such as the induction of regeneration of the spinal cord (Hellal *et al.*, 2011), which might in the future be a potential field of application of the photostatins.

Taken together, the development of reversibly photoswitchable microtubule drugs provides a new powerful tool for the controlled interference with the microtubule cytoskeleton. Considering the ubiquitous importance of microtubules in all eukaryotic cells, the possibility to regulate them locally is instrumental for many experimental designs, but also for microtubule-targeting therapies such as cancer chemotherapy. Though further development of the photostatins will be necessary to make them compatible with cancer treatment, the prospect is very promising, as four combretastatin derivatives, the drugs on which the photostatin design was based, are currently

[#]Correction added on 22 July 2015, after first online publication: the direction of the arrows in Figure 1B has been switched.

undergoing Phase III clinical trials. Today, the drugs developed by Borowiak *et al* (2015) can be applied right away in experimental cell biology, and smaller organisms using the technology that was developed for optogenetic experimentation, while they are yet less adapted for the use in larger organisms or in human medicine. Future developments will be necessary to generate novel chemical scaffolds that will overcome these limitations.

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