Visualization of cytoskeleton with fluorescent microscopy

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1 Abstract

Using certain staining methods, different parts of a cell's cytoskeleton become visible under fluorescent microscope. In the following experiment a primary antibody, an anti-mouse secondary antibody labelled with a fluorescent material and a naturally occurring fluorescent compound is used to visualize the actin and microtubule networks of B16F1 mouse cell cultures with fluorescent microscopy. The fluorescent microscopy revealed visible structural changes to the cell's cytoskeleton in some cases which can be contributed to previously unknown drug treatment in the last hour of the growth phase.

2 Methods

The staining for fluorescent microscopy has been prepared and executed with a slight delay. Prior to the staining the B16F1 cells have grown on glass coverslips with appropriate growth medium at 37 degrees Celsius and 5% CO_2. The cells have been washed to remove excess media and fixed in preparation for staining. Three cultures have been examined of which one have been treated exactly as explained and two may have been treated with a cytoskeletal drug in the last hour of the cells' growth phase.

2.1 Staining

Fluorescent microscopy of the actin and microtubule networks require multiple staining methods. At first the mouse cells have been permeabilized with PBS/0.2% TX100 and to eliminate non-specific binding sites 3% BSA/PBS was used under makeshift humidity chamber. For the second part three fluorescent staining methods have been chosen. The culture has been incubated with Tubulin monoclonal antibody, with Anti-mouse-Alexafluor488 secondary antibody and finally with Phalloidin-Alexafluor594. Between each steps PBS solution has been used to wash and remove excess media. After the staining the coverslips have been mounted to a plastic microscopy film and stored in the dark for four days in a dark room at room temperature.

2.2 Fluorescent microscopy

On the fourth day a fluorescent microscope has been used to have a preview of the stained culture. The fluorescent microscope is connected to a computer with a software able to create adequate pictures of the previewed culture which pictures has been used to determine the drugs used on the mouse cells.

3. Results

The fluorescence microscopy showed a clear image of the cells' cytoskeletal structure. As a result of the staining methods the actin network was visible in red and the microtubule network in green. To have a structured explanation of the drugs effects it is important to first recognize the untreated network and explain why it is. Therefore the first culture to be examined is coverslip B on the laboratory's plastic film. To have a clear view of the cells, only the 100x magnification pictures will be shown.

3.1 Coverslip B

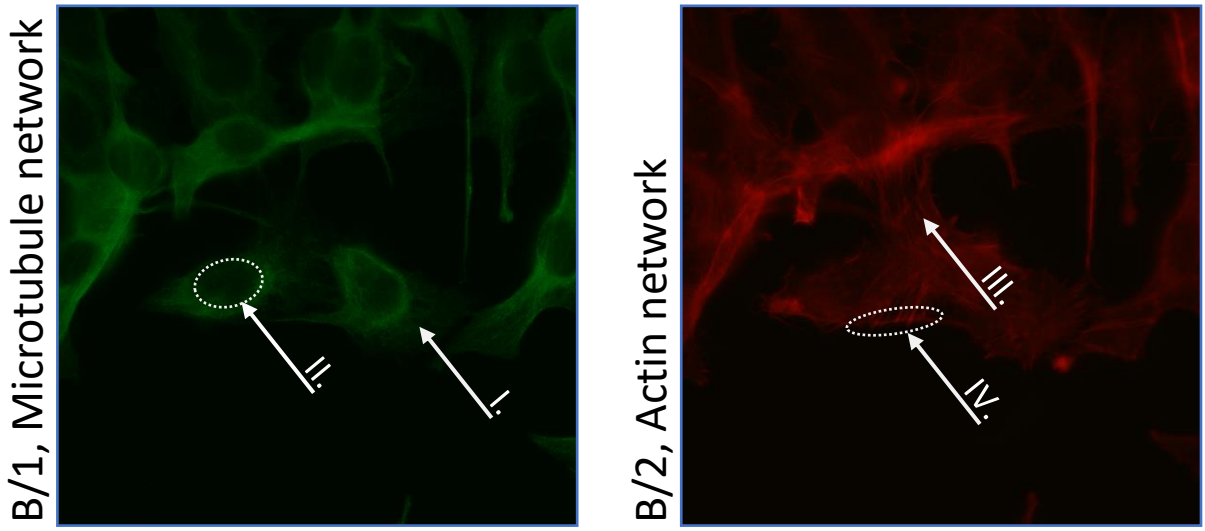

Figure 1. **Coverslip B. B16F1 mouse cancer cells stained with the methods mentioned in 2.1 as seen under fluorescence microscope. B/1**, the microtubule network in green and **B/2**, the actin network in red. The cells in the culture **B** does not show any effect of drug treatment. Proof of this statement is the organized structures that have been pointed out in **I-IV**. In **B/1** the microtubule network forms organized structures across the cell (e.g. **I**.) while being centered around a void or absence of microtubules (e.g. **II**.) which complies with the fact that healthy microtubules' negative ends centers around the Microtubule Organizing Center (MTOC or centrosome). In **B/2** the actin network - similarly to the MT network - forms organized structures in the entirety of the cells like leading edges (e.g. **IV**.) and stress fibers (e.g. **III**.). Also a sign of no cytoskeletal drug exposure is the absence of aggregates.

3.2 Coverslip C

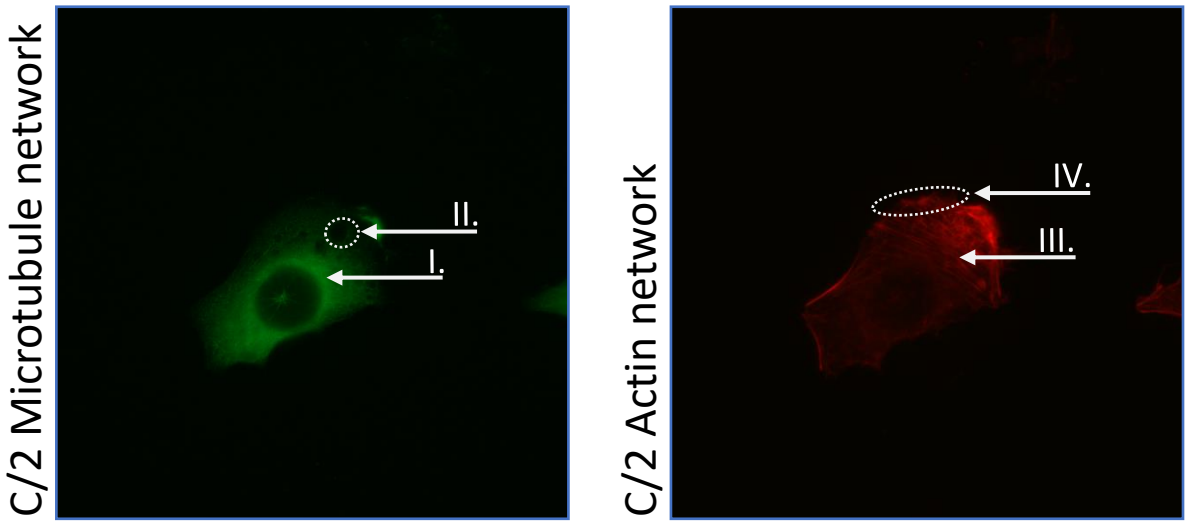

Figure 2. **Coverslip C.** In **C/2** the actin network still possesses the actin specific structures like stress fibers (e.g. **III**.) and leading edges (e.g. **VI.**) similarly to **B/2**. However the MT network in **C/2** looks to be effected with a cytoskeletal drug as it has a denser network or diffused monomers (e.g. **I**.) than **B/1** so much so that smaller organells start to have a distinctive outline (e.g. **II**.).

3.3 Coverslip A

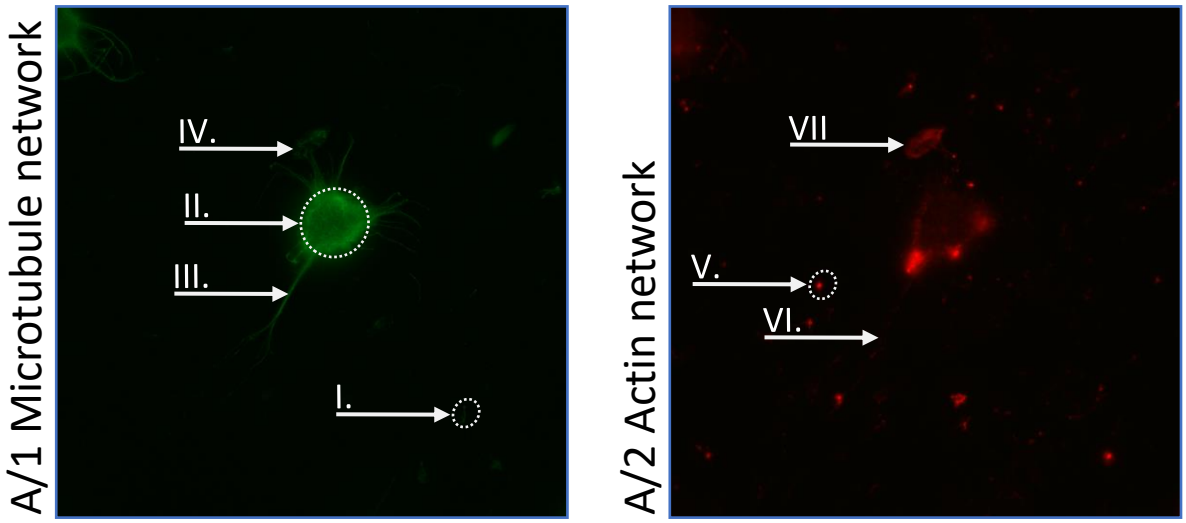

Figure 3. **Coverslip A.** Neither **A/1** nor **A/2** showed similarity to **coverslip B**, numerous differences are present between the two cultures therefore it is evident that culture a has encountered a cytoskeletal drug. The microtubule network has been compromised as it does not form complete structures in the cell but rather has rudimentary highways (e.g. **III**. And **IV**.) and some minor aggregates (e.g. **I**.) while the centrosome appears to be rather disorganized as well (e.g. **II**.). The actin network looks very similarly compromised. The A/2 shows actin aggregates (e.g. **V**.) and matching rudimentary structures (e.g. **VI**. and **VII**.) to the microtubule network.

4 Questions

All of the questions regarding the coverslips and the experiment with the fluorescent microscopy have been based on the pictures shown in *Figures 1-3*. and the observations that are pointed out with Greek numerals.

4.1 Determining which cover slip was treated

Coverslip B was ***untreated*** based on the structural organization and integrity of the microtubules and actin. Cytoskeleton specific structures were found in both microtubules (MTOC) and actin (leading edges and stress fibers) networks.

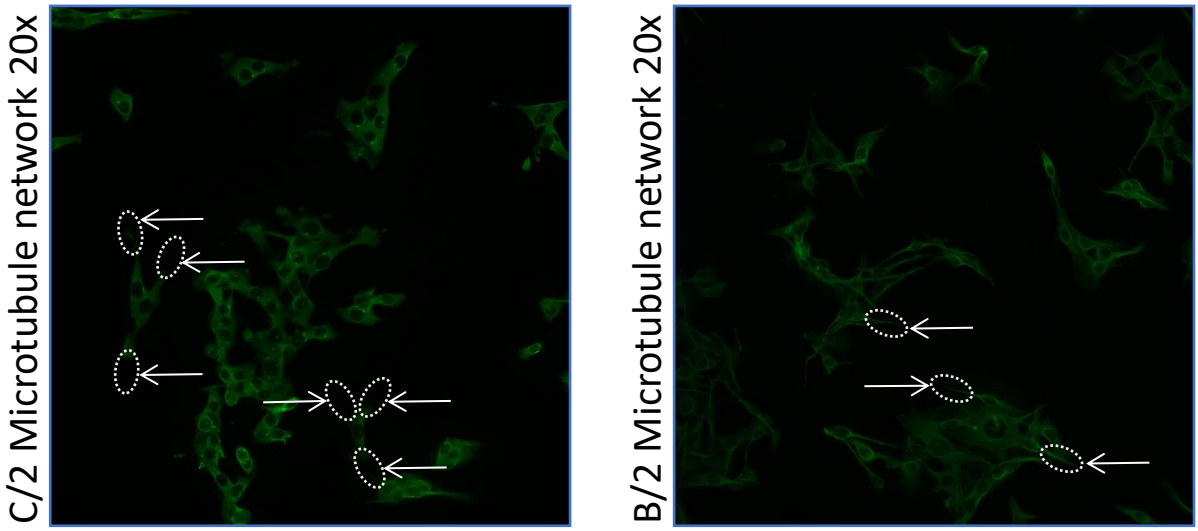

Figure 4. **Microtubule network of Coverslip C compared to B.** Multiple cell structures with 20x magnification shows a visible reduction on **C/2** cells compared to **B/2** untreated cells (some examples shown in the picture).

Coverslip C was ***treated*** with a cytoskeletal drug ***effecting only the microtubule*** network. To determine the cytoskeletal drug it is crucial to understand that the primary antibody works by binding to the alpha and beta tubule monomers as well as to the polymers. However it shows an increased affinity to the polymers. The fluorescent microscopy showed similarities in the network with the untreated MT network in the meaning that the network spread all over the cell with the addition of a blurry denser background inside the cell (*Figure 2*). Combining these information I conclude that the cells in coverslip C has experienced strongly decreased or inhibited polymerization in the last hour of the growth cycle but the

actin and most of the microtubule network has already been built by then hence the complete actin and semi complete microtubule network. It is also notable that existing microtubule networks experience treadmilling when not capped by gamma tubules and the expression of tubule monomers are not directly affected by colchicine. This causes an excess of tubule monomers which the primary antibody can bind to and cause the blurred background. Therefore a putative but plausible theory is that ***colchicine*** or other drugs with the ability to bind to colchicine binding sites like ***SKLB050*** has been used to disrupt the microtubule network.

Coverslip A was ***treated*** with a cytoskeletal drug that ***directly or indirectly affected both actin and microtubule network***. In *Figure 3* it was evident that the more prominent and distinguishable features presented in the microtubule network. The rudimentary highways pointed out in **III**. and **IV**. were stable bundles of microtubules which coupled with the scarce distribution and some aggregates shows a distinct and limited picture of what drugs the cell could have been affected with. Analyzing the actin network shows in *Figure 3* presents actin filaments gather around the stable and MTOC centered microtubules shown in **VI**. and **VII.** while also having aggregates scattered in the cell. This kind of bundling in the MTs and following by the actin network is a known outcome of MT stabilizing drugs namely ***Taxol***. Taxol directly effects the microtubule network however actin forms with the help of Rho GTPases and other resources that are directed by the highways created by the microtubule network therefore Taxol indirectly effects the actin network.

4.2 How different cytoskeletal drugs effect the microtubule network

Colchicine has the ability to bind to microtubules which inhibits intracellular microtubule assembly and polymerization. Depending on how early the colchicine was introduced to the cell the fluorescent microscopy can show minor to major disruptions to the microtubule network and as the microtubule growth is slightly intertwined with the actin growth the actin could be disorganized as well. The earlier the drug was introduced to the cell the less microtubule structures, the more blurred monomers and less actin structures would be present in the system. It is also notable that disruptions in the MT network causes morphological changes.

Taxol binds to beta tubule making the polymer extremely stable. The process disturbs the microtubule catastrophy and rescue dynamic and promotes uncontrollable polymerization without depolymerization. As a result large dense and stable MT bundles form that are scarcely distributed mostly originated from the MTOC still however some aggregates can form as well. Fluorescent microscopy would show long relatively wide rope like formations of green MTs that mostly center around the MTOC but are dense so the MTOC is hardly visible.

Vinca alkaloids bind to the tubule dimers (alpha and beta) preventing polymerization but they also promote depolymerization. After treating the cell with vinca alkaloids it is expected to show significantly decreased microtubule network structures and increased blurry or aggregated spots as the dimers can disperse in the cell but also make aggregates.

4.3 How different cytoskeletal drugs effect the actin network

Cytochalsin binds to the actins barbed (positive) end inhibiting further polymerization while not effecting the pointed (negative) end. This causes a depolymerization of the actin network over time. Depending on the time of introduction to the drug the cell can show some structured elements but over time it disassembles into a blurred picture with slightly denser patchy parts with fluorescent microscopy.

A growth hormone that activates Rho GTPases causes mass reorganization of the actin network. Multiple Rho GTPase like RhoA, Rac1 and Cdc42 are the coordinators of the actins structure and they are known to help creating thick and long stress fibers, an increased number of leading edges and lamellipodia. Fluorescent microscopy would show different actin specific structures increased in length, height and thickness.