Effects of taxol, eribulin and EB1 on microtubules

O. Baghdasaryan, E. Dyuldin, A. Fradkova, D. Garmaeva, A. Yushkevich, P. Zhurlova, B. Nikashin, E. Seregina, N. Dashkevich, N. Gudimchuk

Introduction:

Microtubule dynamics is important for cell division. If the dynamics of microtubules is inhibited by small molecule drugs, cells can no longer divide and they normally die through apoptosis. That is why inhibitors of microtubule dynamics are widely used to stop proliferation of tumor cells. Microtubule dynamics in the cells are also regulated by a number of microtubule-associated proteins, like EB1. These proteins can significantly alter microtubule dynamics and potentially affect their sensitivity to inhibitors.

What is our goal?

Characterize microtubule dynamics in presence of combination of inhibitors and end-binding protein, EB1, in vitro.

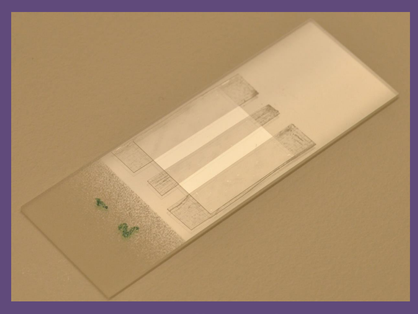
Methods

EB1 purification

1. Lyse E. coli cells
2. Extract soluble proteins
3. Purify EB1 from the extract using Ni-NTA agarose
4. Check the purity using SDS-PAGE
5. Measure the concentration of EB1 using spectrophotometer
6. Aliquot and freeze in liquid Nitrogen

Microscopy

1. Prepare flow chamber with silanized coverslip
2. Wash the chamber with anti-digoxigenin antibodies, incubate for 10 minutes
3. Wash pluronic F127, incubate for 20 minutes
4. Wash microtubule seeds in 0.1 mM GMPCPP
5. Wash the tubulin solution (with/without inhibitors) into the chamber
6. Acquire images using Leica TIRF-microscope
7. Process data using ImageJ Fiji



Microscopy flow chamber

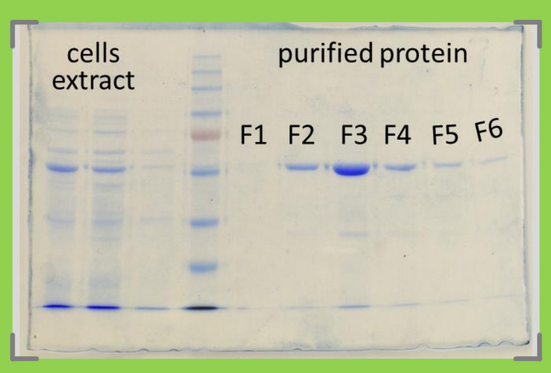
Results

EB1-GFP Purification

1. EB1 protein was purified

Fractions of purified EB1-GFP protein

2. Protein purity was analyzed



SDS PAGE Analysis of protein purification

3. Protein concentration was measured using spectrophotometer

$$C = A_{488} / \varepsilon L$$

$$C = 11.6\ \mu M$$

A_{488} – absorbance at 488 nm
E – extinction coefficient
L – length of optical path

4. After thawing protein crashed out from the solution

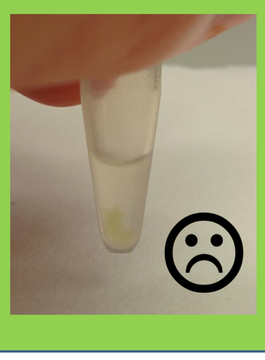
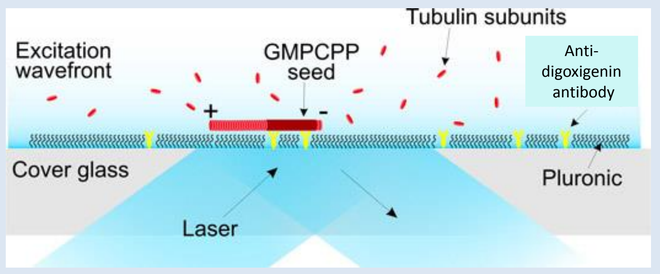


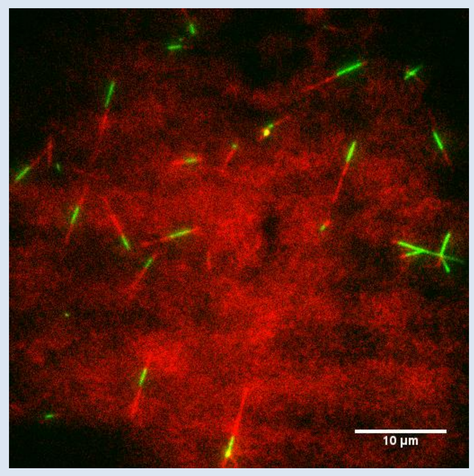
Photo of a tube containing precipitated protein

TIRF microscopy

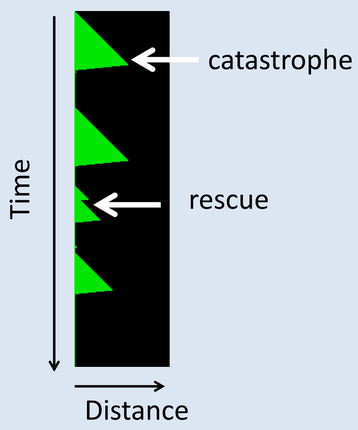
1) Microtubule dynamics were visualized

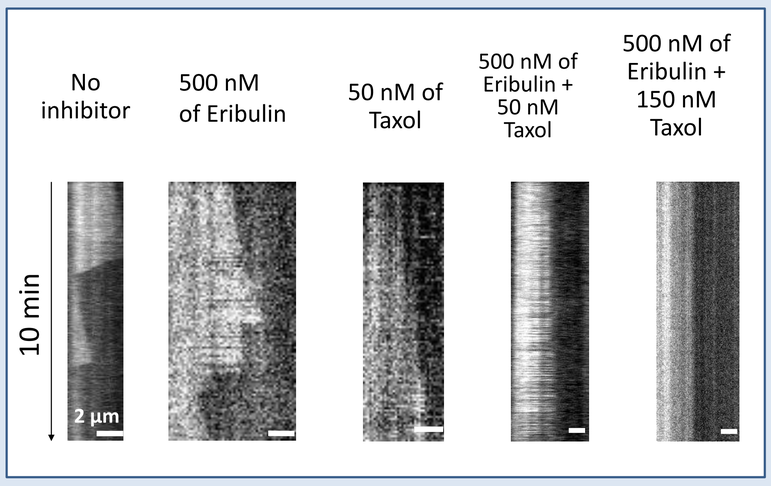


Green – seeds
Red - microtubules

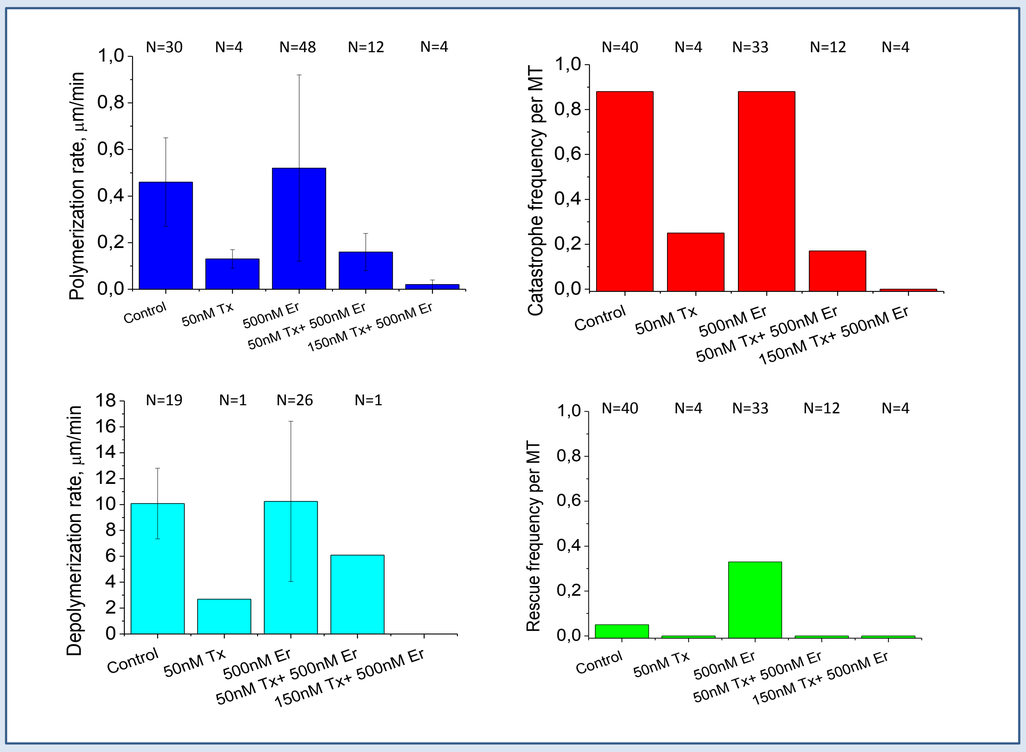


2) Dynamics of microtubules were analyzed





3) The effect of inhibitors was measured



Conclusions

- Eribulin didn't change polymerization rate, depolymerization rate or catastrophe frequency
- Eribulin dramatically increased rescue frequency
- Taxol decreased the rate of polymerization and depolymerization, it also decreased catastrophe frequency
- We didn't notice any effect of taxol on rescue frequency
- 50 nM taxol with 500 nM Eribulin had the same effect on microtubules as 50 nM taxol

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