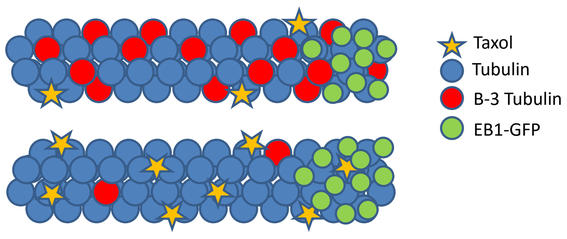
Depletion of β-3 tubulin in microtubule increases sensitivity to the anticancer drug Paclitaxel

Gubanova E., Dobrosmyslov R., Ignatyeva E., Kabanovsky D., Shelepova E., Fomicheva S., Dashkevich N., Nikashin B., Santana E., Seregina E., Gudimchuk N.

Introduction

Microtubules mediate cell division by segregating chromosomes during mitosis. EB1 protein acts as a regulator of their dynamics and sensitivity to Taxol. Taxol is currently one of the most successful anticancer drugs which functions by inhibiting microtubules. Some cells that show resistance to Taxol are found to express β-3 isotype of tubulin. In this work we are testing microtubules sensitivity to various concentrations of Taxol in presence and absence of β-3 tubulin.



Goals

1) Purify EB-1-GFP-6xHis.
2) Deplete β-3 tubulin from microtubules.
3) Check the effect of Taxol on β-3 depleted microtubules in presence of EB-1.

Workflow (methods)

Prepare EB1-GFP

- Express EB-1-GFP-6xHis in *E.coli*
- Lyse *E.coli*
- Add lysate to Ni column and wash unbound proteins
- Elute EB-1-GFP-6xHis with imidazole
- Use SDS-PAGE to check EB-1 purity

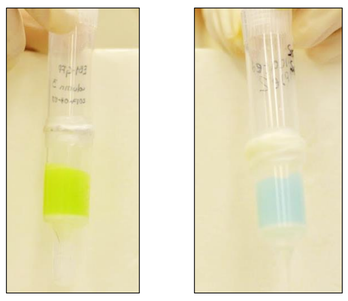
Prepare tubulin

- Coat Protein A magnetic beads with anti-β-3 tubulin antibody
- Wash unbound antibodies
- Incubate beads with tubulin
- Remove beads-β-3 tubulin complex with a magnet
- Collect β-3 depleted tubulin supernatant to use in experiments

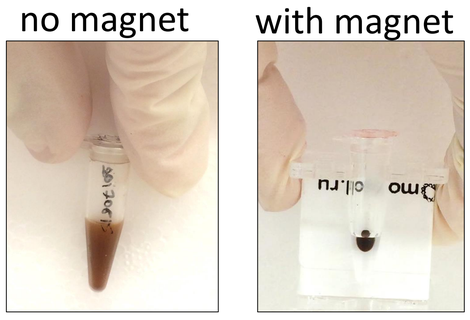
Test microtubules sensitivity to Taxol

- Prepare microtubule chambers in microscope slides
- Prepare microtubule seeds with rhodamine and DIG-labeled tubulin
- Prepare varied Taxol concentrations
- Assemble chambers
- Total Internal Reflection Fluorescence Microscopy (TIRF)

EB-1 bound column | Eluted EB-1 column



no magnet | with magnet

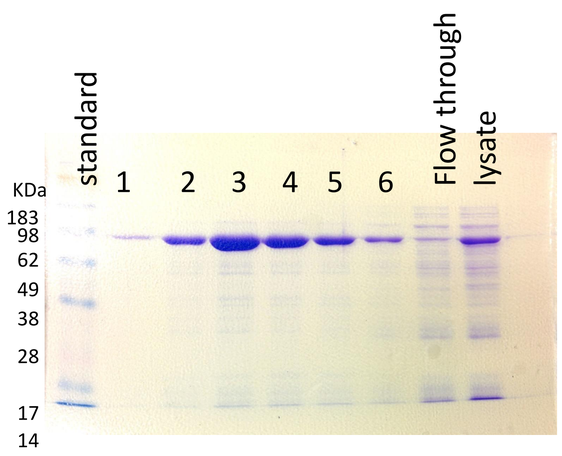


QR code for video!

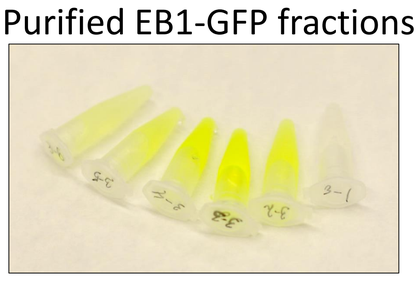
Microscopy chamber

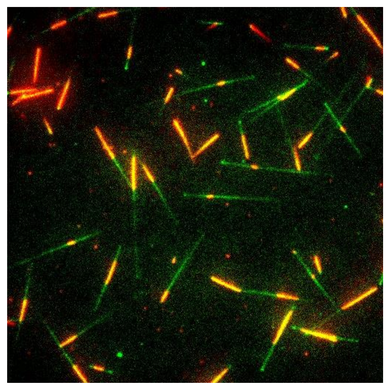
Results

EB-1-GFP-6xHis protein was purified



Purified EB1-GFP fractions



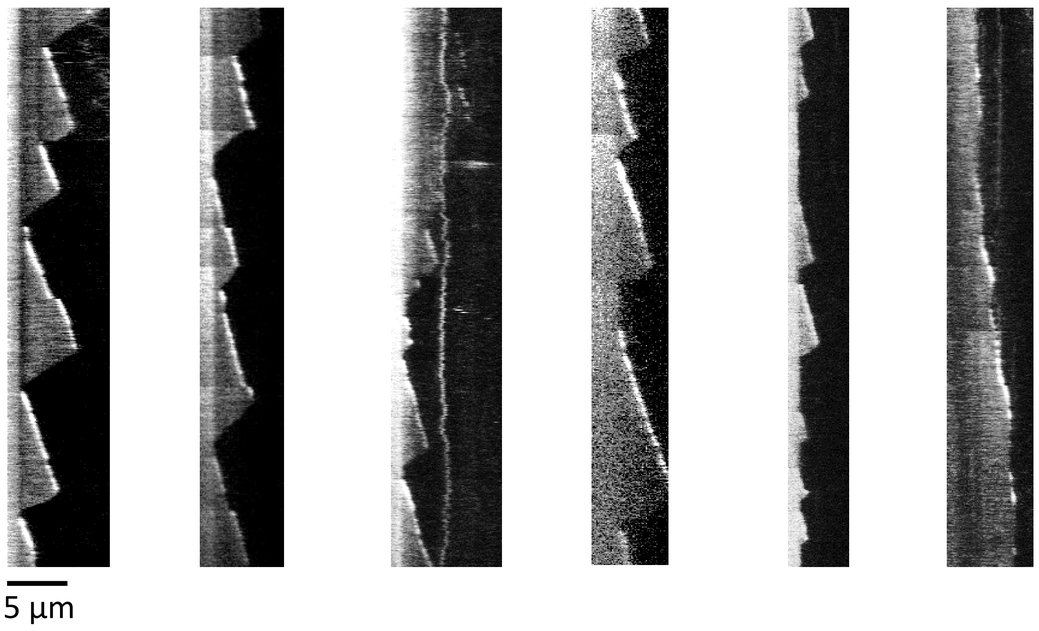


QR code for video!

Summary table

	Depolymerization rate, μm/min	Growth rate μm/min	Catastrophe frequency (1/MT*min)	N of MTs
+β3 No Tx	9,6±4,8	2,1±0,8	0,43	27
+β3 100 nM Tx	8,4±1,9	1,9±0,4	0,54	21
+β3 250 nM Tx	8,0±3,1	1,9±0,6	0,16	20
- β3 No Tx	11,9±5,9	2,0±1,0	0,44	18
- β3 100nM Tx	11,1±5,0	1,4±0,6	0,44	8
- β3 250nM Tx	ND	1,5±0,7	0	23

+β3 No Tx | +β3 100 nM Tx | +β3 250 nM Tx | -β3 No Tx | -β3 100 nM Tx | -β3 250 nM Tx



Kymographs for different experimental conditions were built and processed to extract characteristics of microtubule dynamics

Conclusions

1. Microtubules depleted of β-3 tubulin behave similarly to their non-depleted counterparts.
2. At a 100 nM concentration of Taxol the behavior of both β-3-depleted and non-β-3-depleted microtubules has been the same.
3. At 250 nM no depolymerizations were observed among the β-3-depleted microtubules (expected Taxol effect). On the other hand, depolymerizations were observed among the non- β-3-depleted ones, which supports our initial hypothesis.