NDC-80 INHIBITORS AS POTENCIAL ANTI-CANCER DRUGS

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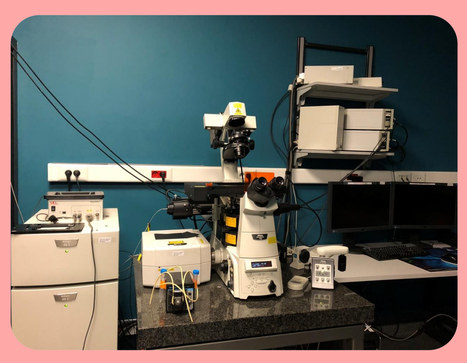
INTRODUCTION

- Cancerous cells divide rapidly
- Microtubules are essential for the segregation of chromosomes in cell division
- NDC-80 binds to microtubules to help in this process
- Molecular docking helps us to select 40 substances that can stop the interaction between NDC-80 and microtubules

GOAL

Our aim is to test 5 out of 40 compounds to stop NDC-80 from binding to microtubules.



METHODS AND MATERIALS

Protein purification

E. Coli lysis to obtain recombinant NDC-80

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Isolate NDC-80-GFP-6His with affinity chromatography

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Collect 10 fractios from the column

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Spectrophotometry to identify the fraction that contains NDC-80-GFP-6His

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SDS-PAGE electroforesis: identify NDC-80 + purity

Microtubules assembly

Microtubules are a polymer of bovine tubulin.

- Unlabeled tubulin
- Rho-labeled tubulin (red fluorescence)
- DIG-labeled tubulin (binds Ab)
- GMPCPP (to stabilize microtubules)

Construction of the flow chamber

- Double-sided tape
- Slides
- Coverslip
- Tubing
- Silicon

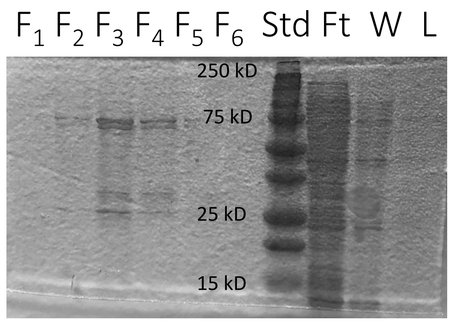
Preparation of compounds solution

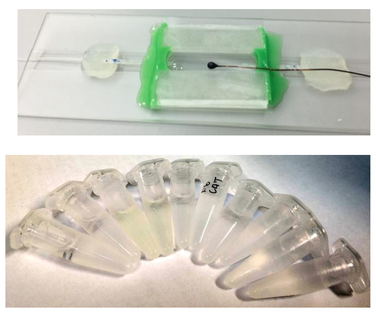
Select 5 inhibitors based on solubility:

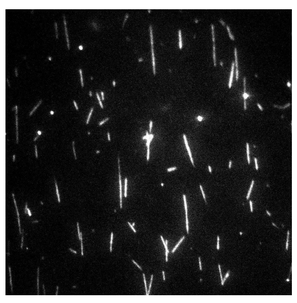
Inhibitor	Solubility	Solute	Final concentration
SMTB 1	12 mol/l	Water	5mM
SMTB 2	0.02 mol/l	DMSO	5mM
SMTB 3	1.14 mol/l	Water	10mM
SMTB 4	0.21 mol/l	Water	10mM
SMTB 6	0.0007 mol/l	DMSO	2.5mM

Microscope

TIRF: Total Internal Reflection Fluorescence to analize our *in vitro* model

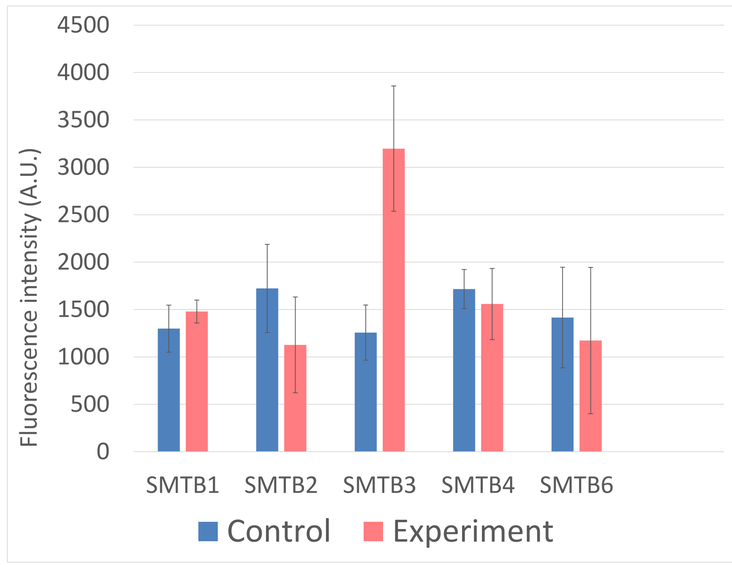






RESULTS

- Protein purification: we collected 10 fractions
- Spectrophotometry: we selected the most concentrated fraction using the formula A= cLE. The best fraction was the fraction 3 with c= 1.136µM
- SDS-PAGE: we loaded the gel with 6 fractions of purified NDC-80, flow-through, wash and lysate. We expected to see two bands (73kDa and 29kDa). We discovered that our protein was sufficiently purified
- Microscopy: we tested five different compounds and determined that the best inhibition effect was observed in SMTB2 compound. We didn't see such a significant difference between the control and 3 of the inhibitors (SMTB1, SMTB4 and SMTB6). Unexpectedly, we observed an increase in binding between microtubules and NDC-80-GFP-6His in the presence of SMTB3 inhibitor. The cause of this response will require additional study.



CONCLUSIONS

- We successfully purified recombinant NDC-80-GFP-6His by affinity chromatography using Ni^{2+} column producing a concentrated solution in fraction 3. It was confirmed by spectrophotometry and SDS-PAGE.
- Labeled polymerized microtubules in the presence of GMPCPP for stability and our recombinant NDC-80-GFP-6His could be seen in the TIRF microscope
- We tested the inhibitors in our *in vitro* system to observe the interaction between microtubules and inhibitor. Interaction is reflected by changes in fluorescence.