Inhibiting the Anaphase Promoting Complex/ Cyclosome: An Innovative Approach for Cancer Chemotherapy

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Abstract

Background: Mitosis-inhibiting chemotherapeutics, such as taxanes, are frequently used in cancer treatment. However, successful therapy is often hindered by drug-resistant tumors, neurotoxicity, and limited supply. In addition, these therapies only target a single cellular protein: tubulin. For these reasons there is high demand for new drugs with different cellular targets. The anaphase promoting complex/cyclosome (APC/C) is a multi-subunit E3 ubiquitin ligase that acts as the master regulator of mitosis by marking key proteins for degradation. Inhibition of the APC/C offers great potential as an alternative target for antimitotic drug development because its inhibition is predicted to directly induce mitotic arrest and apoptosis. The APC/C catalytic core consists of an ANAPC2/ANAPC11 dimer. Homology models of the ANAPC2/ANAPC11 subunits were analyzed *in silico* to identify Hit compounds predicted to bind the cullin domain of ANAPC2, thereby preventing ANAPC11-ANAPC2 interaction and leading to cell cycle arrest. **Hypothesis**: Hit compounds will prevent co-immunoprecipitation of ANAPC2 and ANAPC11. **Methods**: A375 cells were treated with either DMSO, Hit10, or Paclitaxel and incubated for 6 hours. Cell lysates were prepared from mitotically arrested cells and co-immunoprecipitated with ANAPC11 antibody. The resulting Co-IPs were then analyzed via SDS-PAGE and immunoblotting for both ANAPC11 and ANAPC2. The ANAPC2/ANAPC11 for each treatment condition was quantified employing densitometric analysis. The ratios were expressed as % of DMSO control treatment. **Results**: Cells treated with Hit10 or Paclitaxel had decreased ANAPC2/ANAPC11 ratio compared to the DMSO control group. **Conclusions**: Treatment with either Hit10 or Paclitaxel disrupts binding of ANAPC11 to ANAPC2 and shows promise as a putative anti-cancer agent.

Introduction I

- Most anti-mitotic drugs only target a single protein: tubulin
- Taxanes are common anti-cancer drugs that work by inhibiting mitosis
- Taxane effectiveness relies on a functional spindle assembly checkpoint and wild-type tubulin, and is hindered by neurotoxicity, supply shortages, and drug resistant tumors
- Alternative mitotic targets need to be developed
- One possible approach is targeting regulators of mitosis itself

Introduction II

The Anaphase Promoting Complex/ Cyclosome (APC/C)

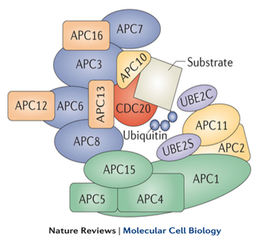


Figure 1. APC/C Structure[1]

- E3 ubiquitin ligase
- Regulates the cell cycle
- Contains 14 different subunit proteins
- Catalytic, TPR arm, co-activator subunits
- Vital for cell proliferation
- APC/C function is required for both mitotic progression and licensing the DNA replication initiation complex

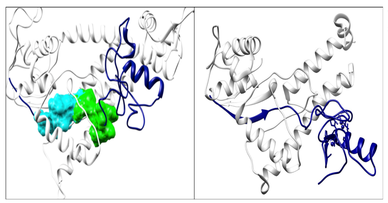


Figure 2. ANAPC2 and ANAPC11 Interaction

- Homology models of ANAPC11 (blue) and ANAPC2 (white)[2]
- In silico docking sites were used to identify Hit compounds predicted to interrupt their interaction[3]

Hypothesis

Hit compounds will bind the ANAPC11 binding site in ANAPC2 and thus prevent co-immunoprecipitation of ANAPC2 and ANAPC11.

Materials and Methods

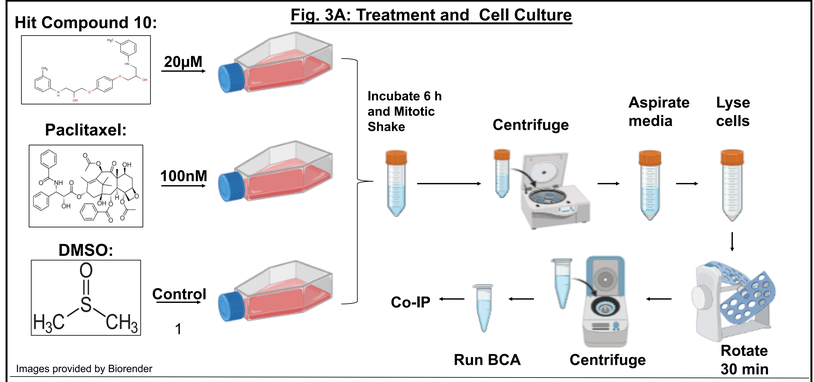


Fig. 3A: Treatment and Cell Culture

Fig. 3B: Co-Immunoprecipitation

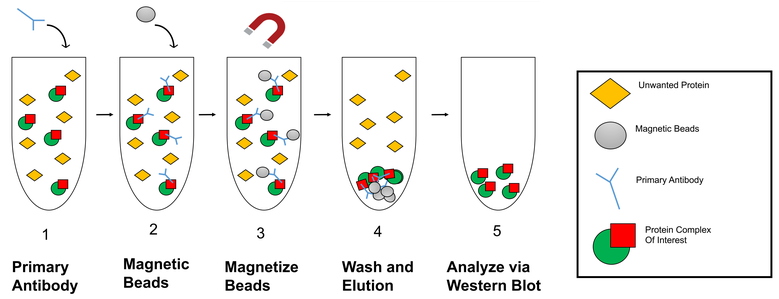
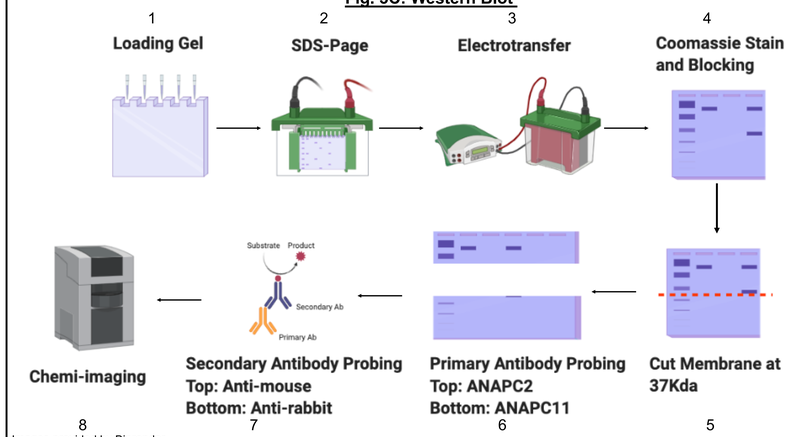


Fig. 3C: Western Blot



Results

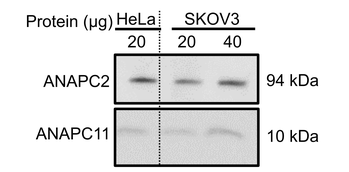


Fig. 4A: Immunoblot Standardization

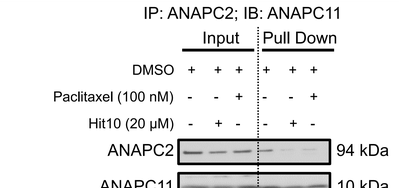


Fig. 4B: Co-IP Standardization

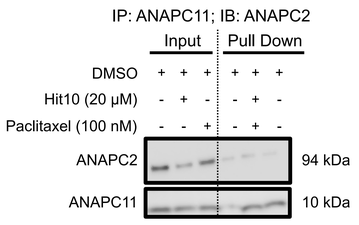


Fig. 4C: Co-IP in for ANAC2 and ANAPC11 Mitotic A375 cells treated with DMSO, Hit10, and Paclitaxel

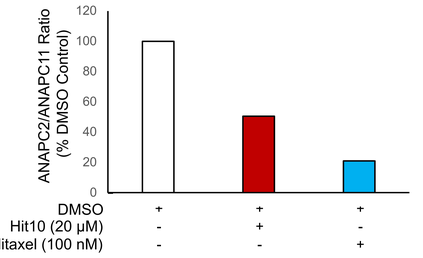
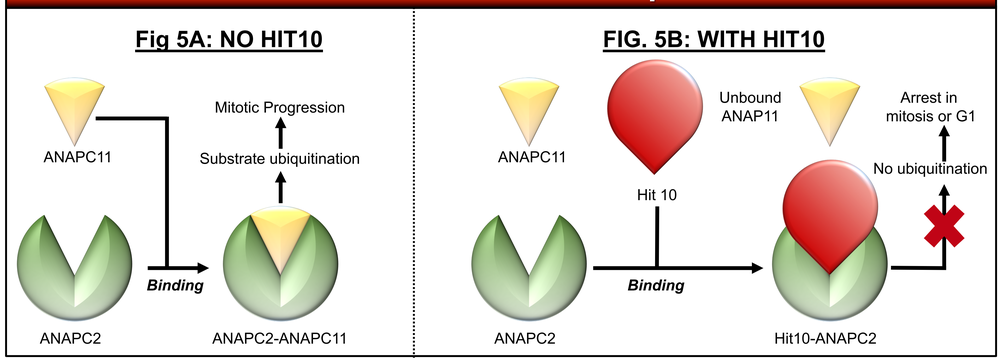


Fig. 4D: Densitometric Analysis of ANAPC2-ANAPC11 Binding

Predicted Mechanism of Hit Compound Action



Conclusions

- Hit10 treatment shows promise in preventing ANAPC2-ANAPC11 binding, thereby possibly leading to disruption of APC/C ubiquitination function resulting in arrest of cancer cells either in mitosis or G1.
- Paclitaxel may have a hitherto unexplored mechanism for inducing mitotic arrest and cancer cell death by disrupting the interaction of ANAPC11 and ANAPC2.

References

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Acknowledgements

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