

From primary microcephaly-associated CPAP as centriole size/number regulator to microtubules-targeting novel chemotype

A Thesis submitted by

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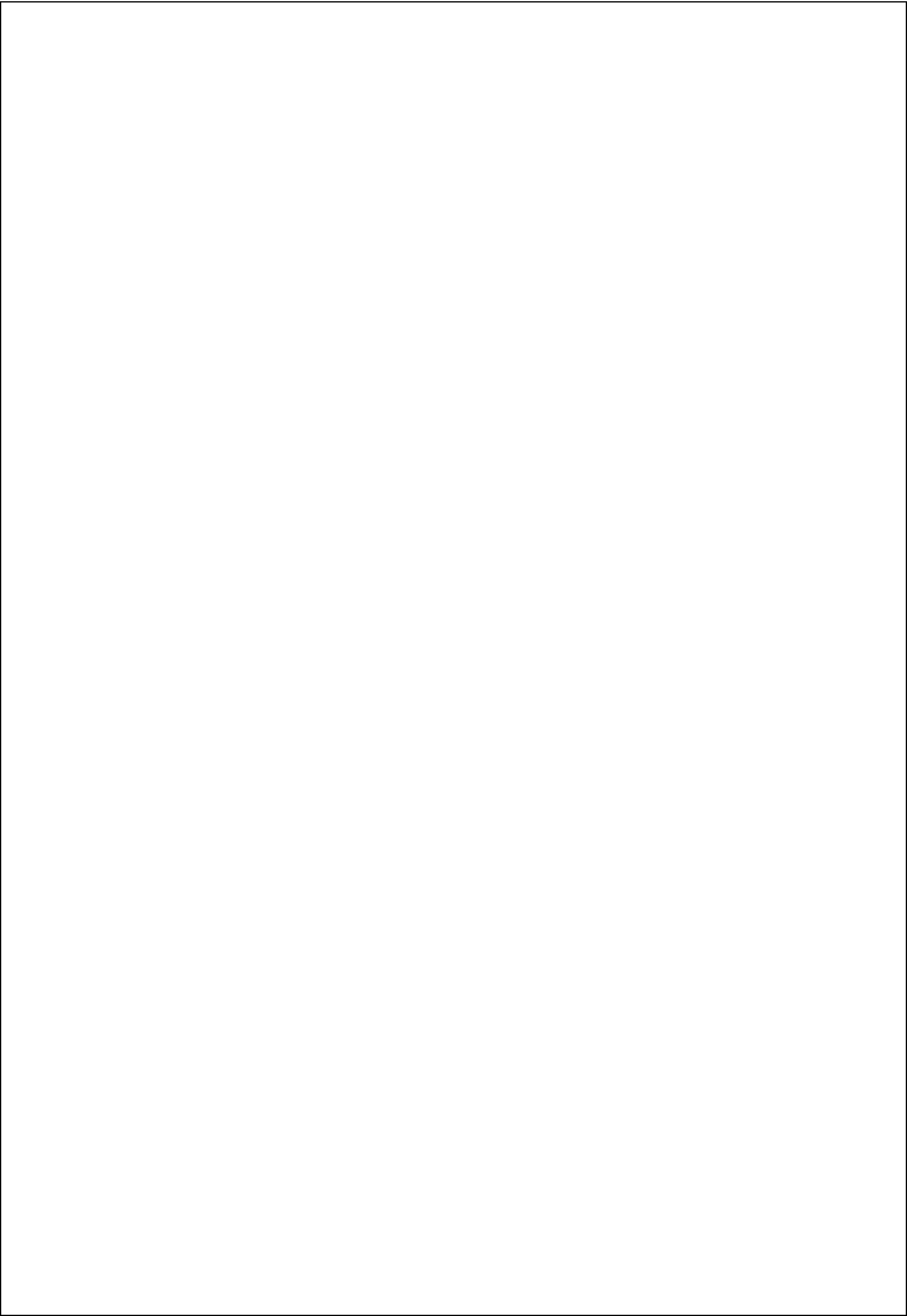


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Summary and Future Directions

MTs are made up of α/β -tubulin dimers and are quite dynamic which allows them to contribute to cell shape, migration and motility. The same MTs also form the structural component of certain cell organelles like centrosomes and cilia. In order to provide structural stability they are modified by specific post-translational events or MT-binding proteins, which provide the requisite robustness and stability to the organelle.

Centrosomes, a MT-organizing cellular machinery consists of a pair of MT-based cylindrical structures called centrioles at their core. The role of centriole protein CPAP as an essential regulator of centriole length has emerged lately. The CPAP protein exhibit conserved regions involved in direct MT binding, which include the N-terminal MT-destabilizing domain, PN2-3, and MT-stabilizing. The C-terminal region of CPAP has another conserved region referred to as the G-box domain which is required for the direct interaction with the core centriole cartwheel protein STIL (involved in CPAP localization and procentriole generation) and centriole elongation factors. Interestingly in neurodevelopmental disorders like autosomal recessive primary microcephaly, single missense mutations in the G-box of CPAP are shown to influence centriole length and brain development. In chapter 3 of this thesis, we utilized the two reported MCPH mutations in the G-box of CPAP i.e. E1235V (EV) and D1196N (DN) to tweeze apart the role of the G-box in influencing centriole length and number. We used HeLa cell lines for our work employing siRNA approach which helped us to answer significant questions however brain-relevant cancer cell lines, stem cells, 3D brain organoids combined with CRISPR gene editing approach can help to harness the advantage of the modern techniques.

We show that CPAP is indeed required for centriole organization as its knockdown by RNA interference affected centriole numbers. Our careful analysis of the effect of MCPH mutations of CPAP G-box shows that EV (MCPH6) caused overly long centrioles with no effect on the centriole number, whereas DN does not affect centriole length, however, causes a significant increase in their number. The present study reports that despite being in the same domain, these two mutations have different effects on CPAP function, thus

highlighting the central role of the G-box in controlling organelle number and size. Both mutations reduced the interaction of CPAP with the core centriole protein STIL, which is also required for recruitment to centrioles. Accordingly, the EV mutant does not localize at centrioles, however, DN centriole localization remains unaffected. This lead us to the identification of the importance of an alternate CEP152-dependent pathway required for CPAP recruitment to the centrioles. We report that the localization of centriole cartwheel protein, SAS6 remains unaltered in CPAP wild-type or mutants suggesting the existence of a core centriole structure in this condition. However, the expression of EV mutant in the complementation experiment causes a significant reduction in the CEP135 levels which is a pinhead protein at the interface of centriole cartwheel and MTs.

This prompted us to analyze the effects of MCPH mutants on protein stability. The molecular dynamic analyses of the equivalent crystal structures of the G-box domain from zebrafish revealed that EV has a stabilizing effect, whereas DN has to stabilize effect on CPAP protein. Finally, we could show that these MCPH mutations cause multipolar spindles and reduce cell viability, thus providing significant mechanistic insights connecting the role of centriole protein in primary microcephaly. Understanding the etiology of MCPH development is a key step for therapeutics. Important recurring functional mutations map to the G-box domain of CPAP which makes it crucial to understand the involvement of this region in CPAP activity. It would be interesting to look at the structural effects of EV and DN on CPAP G-box organization which can explain the differences observed in its functions. In addition, both investigated MCPH mutations substitute negatively charged amino acids for uncharged amino acids, which points towards the involvement of protein interactions and would be interesting to discover novel interacting proteins. Several centriolar proteins found in complexes with CPAP regulate centriole length and would be attractive candidates for gaining mechanistic insights and their possible involvement in the CPAP centriole localization and functioning. Future research could focus on identifying interacting proteins and signaling pathways involved in CPAP-mediated regulation of centrioles. Characterizing the relationship between CPAP and MT dynamics by elucidating the mechanisms by which CPAP impacts MT polymerization, depolymerization, and stability will enhance understanding of the relationship.

Microtubule dynamics is successfully targeted by many anticancer drugs. The MT-stabilizing drug paclitaxel is widely used in chemotherapeutics in many types of cancer treatments including lung, ovarian and breast cancers. However, many types of cancers develop resistance to paclitaxel or do not respond to treatment due to modifications in

tubulin isotypes. Therefore, it is imperative to identify novel chemical scaffolds which provide flexibility for designing new drugs for chemotherapeutics. In chapter 4 of this thesis work, we identified a novel MT-targeting chemotype containing S-aryl dithiocarbamate moiety. So far only a handful of S-alkyl-based dithiocarbamates have been investigated for their anticancer activities, hence the study provides a novel S-aryl dithiocarbamate scaffold with promising drug-like properties. The *in silico* studies confirm the drug-likeness and acceptable range of ADMET properties of the synthesized S-aryl dithiocarbamate-based compounds. Structure-activity relationship allowed us to narrow it down to a lead compound (compound 1) with an IC_{50} value of $0.432 \pm 0.138 \mu M$. We provided a mechanistic understanding of the antiproliferative activity of compound 1 in cancer cells. We observed MT stabilizing effects of compound 1, which activated the p53-p21 dependent cell cycle arrest in the G₂/M stage and cell apoptosis. This work provides a novel chemical scaffold with the ability of diverse structural flexibility which can be further exploited to enhance its antiproliferative activity. Our molecular docking analysis has led us to gain insights into its possible orientation and interaction in the β -tubulin binding pocket. This will provide a framework to further modify the parent scaffold to enhance its specificity and potency as an anticancer drug. Future research can explore the synthesis and characterization of dithiocarbamate based small molecules or peptides that selectively modulate MT dynamics. Evaluating the efficacy, specificity, and safety profiles of these compounds in relevant disease models, with the ultimate goal of translating these findings into potential therapeutic interventions would also be interesting.

