

Studies on Proteins Involved in Cell Death/Survival Pathways

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DOCTOR OF PHILOSOPHY

by

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Chapter 5

Conclusion and Future Scope

Autophagy is a cellular process with a primary role as a recycling pathway for damaged organelles and long-lived proteins, crucial for maintaining nutrient homeostasis and ensuring quality control within the cell. It's closely intertwined with another form of programmed cell death called apoptosis. These two mechanisms, autophagy and apoptosis, are pivotal in various cellular processes, including development, aging, and regular cellular functions. However, understanding the intricate interplay between them in pathophysiological contexts, such as cancer, neurodegenerative disorders, cardiac abnormalities, and infectious and inflammatory diseases, remains a challenge. However, autophagy's function can be context-dependent, shifting from a "cytoplasmic turnover process" to a "tumor suppressor" and even a "tumor promoter" process as the physiological stress within the cellular microenvironment evolves. In the context of cancer, heightened autophagy can lead to chemotherapy resistance and intolerance, sparking interest in exploring the interplay between autophagy and apoptosis. The full understanding of these two forms of cell death remains a topic ripe for extensive exploration. Despite the growing interest in unraveling the complex synergy between autophagy and apoptosis, our understanding is still in its early stages, providing only partial insights into key regulators like Beclin1. In cancer, as cellular stress escalates, Beclin-1-mediated autophagy takes on a hierarchical role, transitioning from a tumor suppressor to a promoter of tumorigenesis and contributing to chemotherapeutic resistance. There is

emerging evidence suggesting the potential of modulating autophagy for cancer therapy (Figure 5.1). Beclin-1 and Bcl-2 interaction is one of the key regulatory interactions in this interplay, shaping physiological and pathophysiological processes.

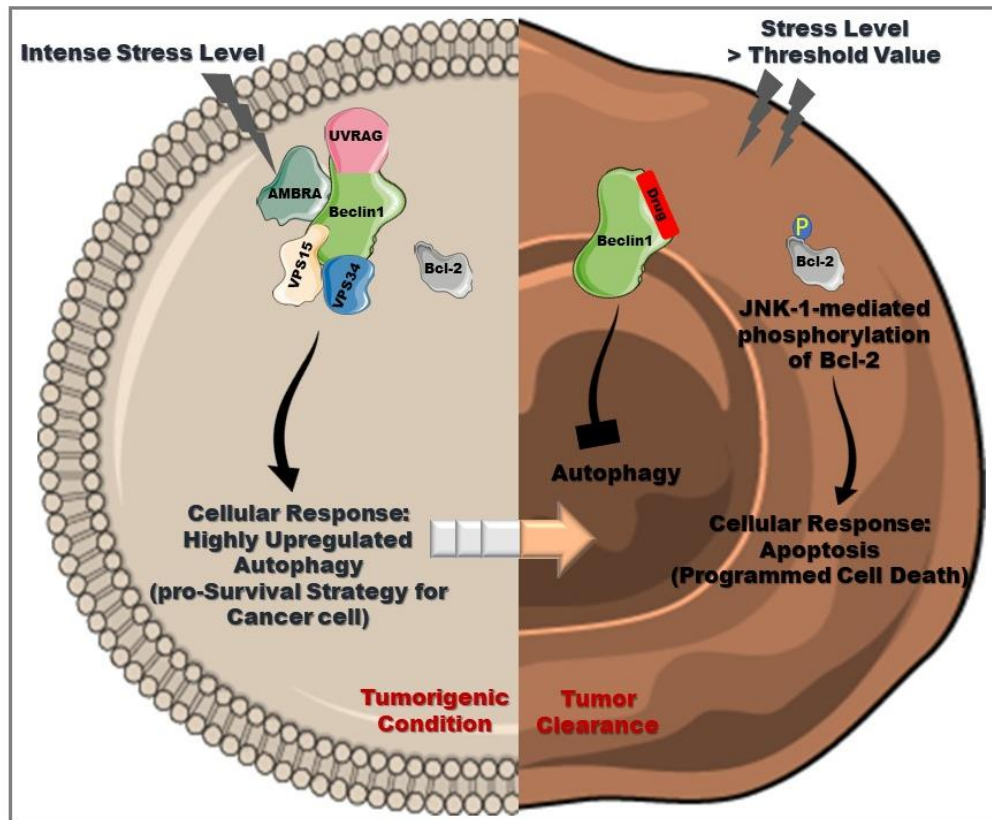


Figure 5.1. Understanding the cross talk between autophagy and apoptosis and regulating the Beclin-1 and Bcl-2 interaction. The interaction between these two proteins plays pivotal role and targeting this key interaction may help in understanding the involvement of autophagy modulators in cancer therapeutics.

In the chapter 1 of the thesis, we have done extensive literature survey and presented the scope of work. Initially, we tried to delve into the intricate relationship between autophagy and apoptosis, with a particular focus on Beclin1's significance in health and disease. We also tried to identify the gaps in the current literature and outline the scope of ongoing work in this field. Autophagy and apoptosis are fundamental processes of programmed cell death present in all eukaryotic cells. However, in the context of the

heightened physiological stress within the tumor microenvironment, autophagy experiences uncontrolled upregulation. Existing literature suggests that inhibiting this upregulated autophagy in cancer cells can potentially trigger apoptosis, ultimately leading to tumor clearance.

Few studies have aimed to design autophagy inhibitors by targeting specific elements such as Beclin-1 or Bcl-2 in isolation. To address the limitations associated with the availability of small, potent autophagy inhibitors, our approach involves an extensive computational effort which we have presented in the chapter 2 of the thesis, to repurpose FDA-approved drugs from the ZINC database (Figure 5.2). The primary objective is to inhibit the interaction between Beclin1 and Bcl-2, thereby curbing cellular autophagy and promoting apoptosis in the tumor microenvironment. Out of the large number of FDA-approved drugs assessed in our computational work, we have identified three promising candidates: Ponatinib, Simeprevir, and Nilotinib. These drugs demonstrated good binding energy and favorable interactions with the BH3 domain of Beclin1, as confirmed through various methods, including molecular docking, Lipinski's filter, MD simulation, and MM/PBSA. While our computational results indicate the potential of these drugs as potent autophagy inhibitors and inducers of apoptosis in the tumor microenvironment, further experimental validations are currently underway to substantiate these *in-silico* findings. While the precise mechanisms by which these drugs inhibit autophagy and activate apoptosis are not yet fully understood, our computational study on targeting the Beclin1-Bcl-2 protein-protein interaction using FDA-approved drugs paves the way for critical *in vitro* and *in vivo* investigations. This research may ultimately offer valuable insights into protein dynamics and play a pivotal role in the realm of drug discovery.

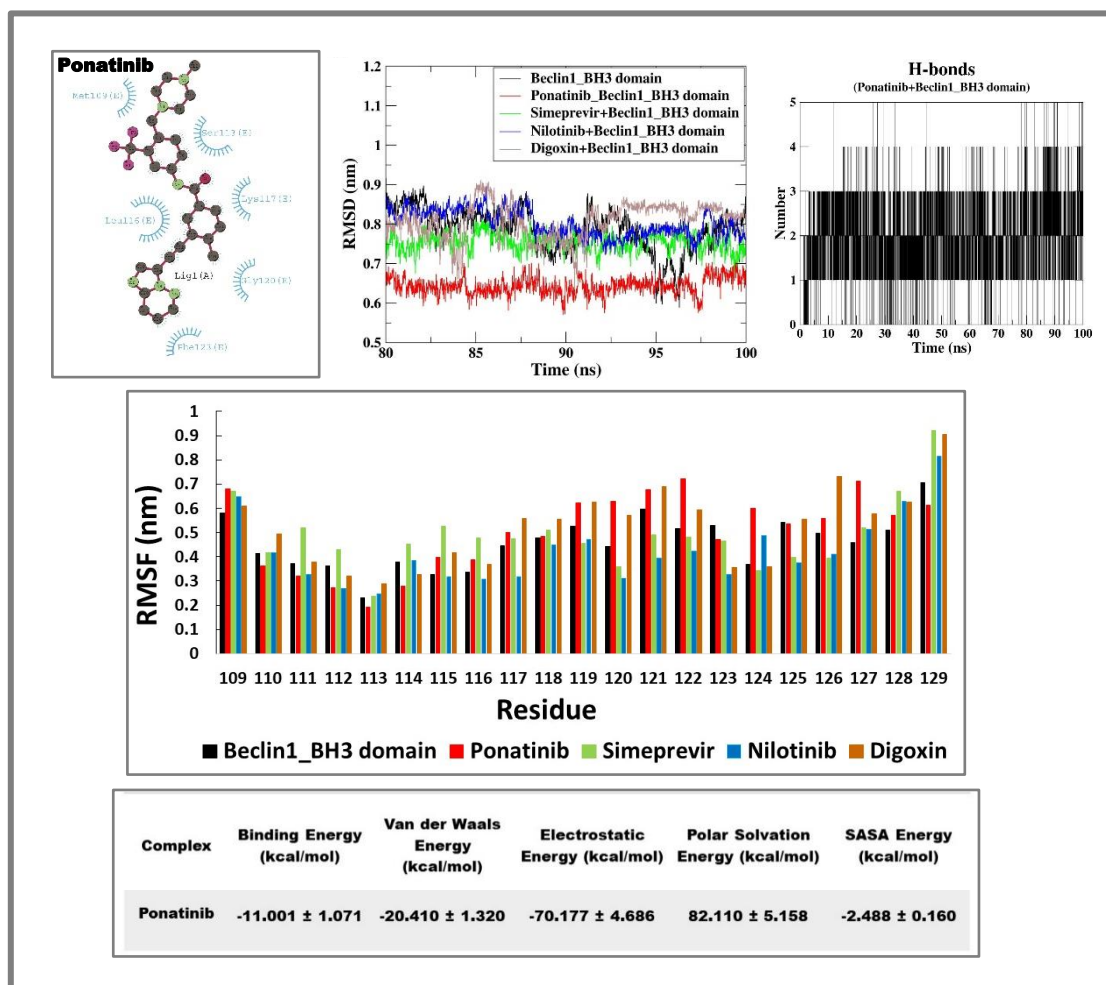


Figure 5.2. Computational work favouring the drug Ponatinib as a potential molecule to bind with the surface of BH3 domain of Beclin-1. Compilation of the key conclusion from the work mentioned in the second chapter which involved repurposing of FDA-approved drugs as autophagy inhibitors in tumor cells. The compiled result shows the behaviour of Ponatinib in potentiating the binding interaction with the BH3 domain of Beclin-1.

Despite the growing interest in unraveling the complex synergy between autophagy and apoptosis, our understanding is still in its early stages, providing only partial insights into key regulators like Beclin1. In cancer, as cellular stress escalates, Beclin1-mediated autophagy takes on a hierarchical role, transitioning from a tumor suppressor to a promoter of tumorigenesis and contributing to chemotherapeutic resistance. There is emerging evidence suggesting the potential of modulating autophagy for cancer therapy. However, further

research is needed to explore the applicability of these insights in developing innovative chemotherapeutic strategies.

Cancer cells often employ extensive autophagy to cope with high metabolic stress, making the inhibition of this heightened autophagic flux an appealing target for cancer therapy. Autophagy-related gene 4A (ATG4A) plays a crucial role in autophagy, and inhibiting its activity could potentially aid in clearing tumors. To address this problem in the chapter 3 of the thesis, we tried to identify candidate drugs from the large number of FDA-approved drug pool, a subset of the Zinc database, that can act as ATG4A inhibitors with anti-cancer properties. We employed a Computer-Aided Drug Design (CADD) approach, utilizing virtual screening tools like Raccoon and MGLTools-1.5.6. Our investigation led us to identify Lumacaftor as a potent inhibitor of ATG4A based on a comprehensive range of computational methods, including molecular docking, MD simulation, and MM/PBSA. This drug demonstrates potential as a strong candidate for an anti-cancer agent. However, it's important to note that the effectiveness of Lumacaftor as an ATG4A inhibitor for anti-cancer therapy requires further validation and investigation.

A few computational studies have expanded our understanding and facilitated the development of potential anti-autophagy or anti-cancer drugs by screening existing FDA-approved medications. While earlier studies have already reported a few ATG4A inhibitors, our current research aims to broaden the scope of chemical discovery for novel autophagy inhibitors (Figure 5.3). We focused on a diverse set of FDA-approved drugs from the ZINC database, including Lumacaftor, Benzoyl, Dolutegravir, Imatinib, Samsca, and Mepron. Through careful consideration of various parameters, such as RMSD (backbone and ligand), RMSF (in relation to both the complete residues of the drug complex and only the active site residues), Rg, and MM/PBSA, Lumacaftor consistently emerged as the top performer among the drug complexes. Therefore, our study strongly suggests that Lumacaftor holds promise as

a potent candidate for inhibiting the function of ATG4A and could serve as a remarkable drug for further investigation.

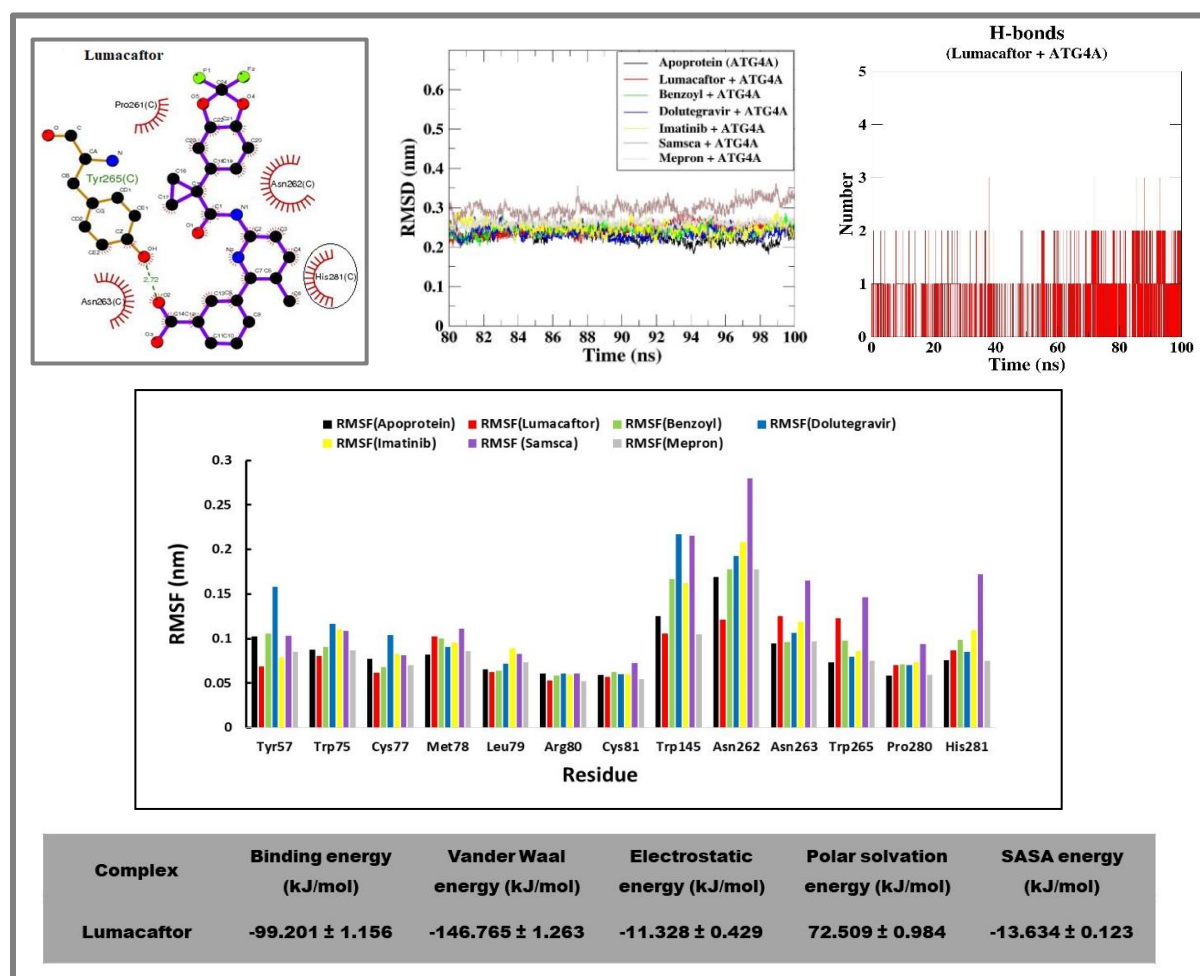


Figure 5.3. Illustration of the outcome of computational work, highlighting Lumacaftor as a promising candidate for binding with the active site of ATG4A. This data compilation consolidates the primary findings from the research detailed in the third chapter, which focused on virtual screening and the repurposing of FDA-approved drugs sourced from the Zinc database. The objective was to identify potential autophagy inhibitors by targeting ATG4A Cysteine Peptidase. The compiled results showcase Lumacaftor's potential as a novel anti-cancer molecule, specifically by enhancing its binding interaction with the enzyme ATG4A.

Our ongoing research contributes to the development of more effective and specific drugs with the potential to inhibit the autophagy-associated ATG4A protein. This, in turn, has

the potential to advance drug discovery in the field of cancer biology, offering promising avenues for anti-cancer therapy.

The interplay between Beclin-1 and Bcl-2 is critical in regulating two vital processes in cells: autophagy and apoptosis. We have presented this regulatory interaction in detail using *in vitro* experiments in the chapter 4 of the thesis. Bcl-2 typically inhibits autophagy by interacting with Beclin1, but disrupting this interaction can shift the balance towards either autophagy or apoptosis, depending on the context. In our previous study mentioned in the chapter 2 of the thesis, Ponatinib, a tyrosine kinase inhibitor, was found to effectively bind to the interface of BH3 domain of Beclin-1 protein. This led to further investigations on how Ponatinib regulates the interaction between Beclin-1 and Bcl-2 and affects autophagy and apoptosis in MCF-7 breast cancer cells. The results suggest that Ponatinib can shift the balance from autophagy to apoptosis in these cells, a pathway that leads to cell death and could be beneficial in clearing cancer cells (Figure 5.4).

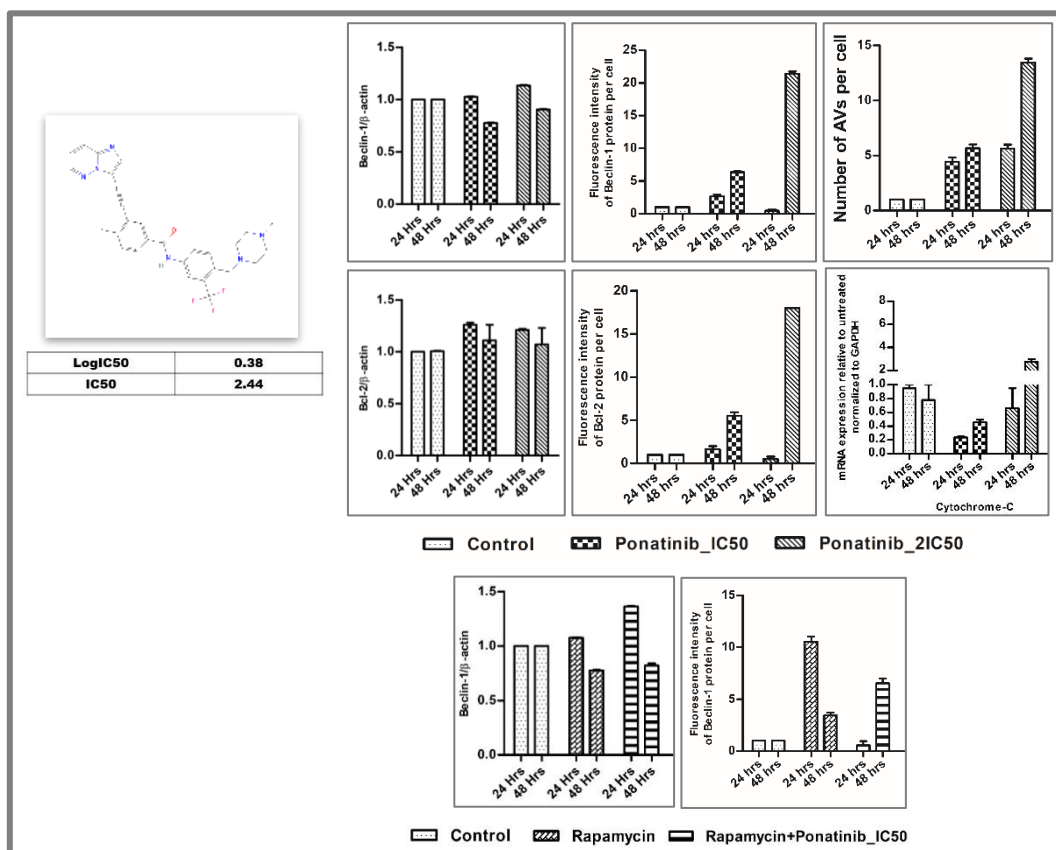


Figure 5.4. Illustration of the outcome of our *in vitro* work, highlighting the effect of Ponatinib on regulating the interaction between Beclin-1 and Bcl-2 and synergistic effect of Rapamycin over Ponatinib. This key data represents the potential behaviour the drug Ponatinib on regulation of Beclin-1 and Bcl-2 interaction, which is compiled in the chapter 4 of the thesis. The compiled results showcase that Ponatinib likely has the tendency to initially upregulate the autophagy and subsequently to inhibit the autophagic flux and to trigger apoptosis in the breast cancer cell. The autophagy inducer drug Rapamycin shows synergistic effect over Ponatinib.

These findings indicate a dynamic modulation of autophagy in response to Ponatinib treatment, reflecting the intricate and time-dependent nature of the autophagy process in the context of the Bcl-2 protein. Our computational and experimental data provide insights into how Ponatinib may be effective in treating cancer by influencing the interaction between Beclin-1 and Bcl-2. Subsequent experiments validated that Ponatinib effectively influences the balance between autophagy and apoptosis in breast cancer cells, suggesting its potential as a therapeutic intervention. This approach could help overcome challenges related to chemotherapeutic resistance and enhance tumor clearance.