Preventing Senescence Associated Age-Related Diseases by Targeting Anti-apoptotic Proteins BCL-XL and BCL-2 Using ICM Molsoft

Yareli Moreno • Isabel Molina • William Munroe • HSI SAGE Conference

Abstract

The accumulation of unhealthy cells in organisms that have entered cellular senescence, which is irreversible, have been known to cause many age-related diseases; due to their anti-apoptotic properties. As aging increases an individual's risk of developing chronic diseases, it is possible that targeting aging by regulating senescence pathways can delay the on start of senescence and enhance the health of individuals. A group of drugs known as senolytics have been recognized for their ability to promote apoptosis in senescent cells by targeting anti apoptotic proteins. In this experiment the antiapoptotic proteins BCL-xL and BCL-2, found in both *planaria* and humans, were analyzed to determine if senolytic drugs ABT-737 and ABT-263 (Navitoclax) could interact and inhibit their anti-apoptotic mechanisms; to induce apoptosis of the senescent cells. *Planarian* proteins BCL-xL and BCL-2, were analyzed i*n silico.* The Planmine protein sequence database and *Dugesia japonica* Nucleotide BLAST from the U.S. National Library of Medicine was used along with the computer application Robetta in the creation of *de novo planarian* protein structures. The structures were used in protein-ligand docking computational simulations using ICM Molsoft. ABT-737 in complex with *planarian* BCL-xL did not yield favorable binding energy values in kcal/mol; thus, ABT-737 unsuccessfully bound with *planarian* BCL-xL. However, ABT-263 yielded negative binding energy values in kcal/mol; thus, ABT-263 bound successfully to *planarian* BCL-xL and BCL-2. These findings indicate that Navitoclax can potentially bind to BCL-xL and BCL-2 proteins and remove senescent cells in *planarian* models.

Introduction

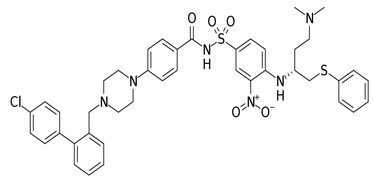
Senescence is a natural process that occurs, in cells, as a result of aging (Kang, 2019). As cells age, they can become senescent through a variety of complications, during cell replication, which include telomere attrition, DNA damage, and oncogenic mutations, among others (von Kobbe, 2019). In addition, a strong correlation between aging and diseases has been observed in previous studies. These studies indicate that it is possible that the accumulation of senescent cells contributes to the decreasing function of physiological processes; thus, leading to disease (Childs 2015). Examples include cancer, diabetes, osteoporosis, cardiovascular disease, stroke, Alzheimer's disease and related dementias, and osteoarthritis (U.S. Department of Health and Human Services, 2021).

In addition, as aging increases, the process by which senescent cells can be properly removed becomes impaired (Kang, 2019). This mechanism is called apoptosis and it is an essential mechanism in the body that promotes programmed cell death for damaged cells. Senescent cells are characteristic for expressing anti apoptotic proteins more abundantly than non-senescent cells (Kang, 2019). A resistance to the normal mechanisms of apoptosis have resulted from the accumulation of these cells in organisms; thus, increasing the risk for disease (2015). Furthermore, these anti apoptotic proteins located in senescent cells can be targeted by senolytic drugs which selectively clear senescent cells (Kirkland 2020). This study aims to analyze the interactions between senolytic drugs and anti-apoptotic proteins to possibly eliminate the number of senescent cells in an organism; thus, preventing disease and extending the lifespan of an organism.

Methodology

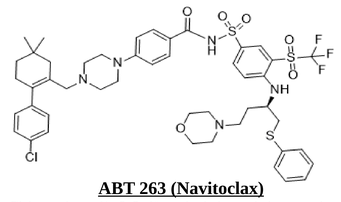
Human BCL-xL and BCL- 2 files were obtained from RCSB PDB (http://www.rcsb.org/) repository and used in ICM molsoft (https://www.molsoft.com/about.html). Docking was performed in ICM molsoft in order to analyze the binding interaction between the anti-apoptotic drug ABT-263 and the *planarian* proteins BCL-xL and BCL-2 pdb files obtained from RCSB. Homologous *planarian* proteins for BCL-XL were searched for using Planmine, a *planarian* protein data bank (https://planmine.mpibpc.mpg.de/planmine/begin.do). A blast search was performed in order to search for *planarian* proteins that were similar to the human form BCL-xL obtained from RCSB PDB. Homologous planaria proteins for BCl-2 were searched for using *Dugesia japonica* Nucleotide BLAST from U.S. National Library of Medicine, where DNA Sequences were generated. DNA sequences were used in the BioModel Transcription and Translation Tool and were transcribed to yield RNA sequences and eventually single-letter amino acid sequences for BCL-2. P*lanarian de novo* protein structures were generated using Robetta, a protein modeling software which is based of off Rosetta by Barker lab (robetta.bakerlab.org). Generated *planarian* protein structures were compared to human protein structures.

Results



ABT 737

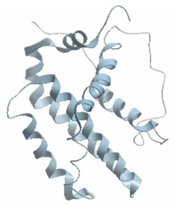
4-{4-[(4′-Chloro[1,1′-biphenyl]-2-yl)methyl]piperazin-1-yl}-N-(4-{[(2R)-4-(dimethylamino)-1-(phenylsulfanyl)butan-2-yl]amino}-3-nitrobenzene-1-sulfonyl)benzamide



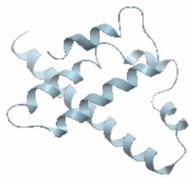
ABT 263 (Navitoclax)

4-(4-{[2-(4-Chlorophenyl)-5,5-dimethyl-1-cyclohexen-1-yl]methyl}-1-piperazinyl)-N-[(4-{[(2R)-4-(4-morpholinyl)-1-(phenylsulfanyl)-2-butanyl]amino}-3-[(trifluoromethyl)sulfonyl]phenyl)sulfonyl]benzamide

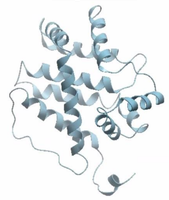
Human BCL-2 Protein



***Planarian* BCL-2 Protein Sequence 2**



***Planarian* BCL-xL Protein Sequence 6**



Human BCL-xL Protein

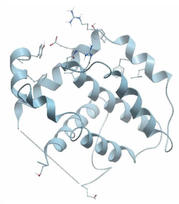


TABLE 1: ABT 737 and *Planarian* BCL-xL binding pocket number vs binding energy

Protein Sequence	Pocket number	Binding Energy (kcal/mol)
Planarian Protein sequence 3	1	-0.1520
Planarian Protein sequence 4	1	0.6403
Planarian Protein sequence 5	1	-0.4154

TABLE 2: ABT 737 and Human BCL-xL protein binding pocket number vs binding energy

Protein Sequence	Pocket number	Binding Energy (kcal/mol)
Human BCL-xL	1	0.3647

TABLE 3: ABT 737 and *Planarian* BCL-2 binding pocket number vs binding energy

Protein Sequence:	Pocket number	Binding Energy (kcal/mol)
BCL-2 *Planaria* Protein sequence 1 Trail #1	1	-0.7819
BCL-2 *Planaria* Protein sequence 1 Trail #2	1	270.5
BCL-2 *Planaria* Protein sequence 1 Trail #3	1	-1.113
BCL-2 *Planaria* Protein sequence 1 Trail #4	1	1.166
BCL-2 *Planaria* Protein sequence 2 Trail #1	1	0.829
BCL-2 *Planaria* Protein sequence 2 Trail #2	2	0.5033

TABLE 4: ABT 263 and Human BCL-xL protein binding pocket number vs binding energy

Protein	Pocket number	Binding Energy (kcal/mol)
Human BCL-xL	1	-9.573
Human BCL-xL	2	-10.81
Human BCL-xL	3	-9.829
Human BCL-xL	4	-9.538

TABLE 5: ABT 263 and *Planarian* BCL-xL proteins Protein Pocket #1 vs binding energy

Protein Sequence	Pocket number	Binding Energy (kcal/mol)
BCL-xL Planarian Protein Sequence 1	1	-10.04
BCL-xL Planarian Protein Sequence 2	1	-11.01
BCL-xL Planarian Protein Sequence 3	1	-10.81
BCL-xL Planarian Protein Sequence 4	1	-10.02

TABLE 6: ABT 263 and *Planarian* BCL-2 binding pocket number vs binding energy

Protein Sequence	Pocket number	Binding Energy (kcal/mol)
BCL-2 *Planaria* Protein sequence 2	2	-9.468

TABLE 7: ABT 263 and Human BCL-2 protein binding pocket number vs binding energy

Protein Sequence	Pocket number	Binding Energy (kcal/mol)
Human BCL-2	1	-10.76

Literature Cited

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Conclusions

A successful binding energy value in Kilocalories/mole is a negative value, and the values that have lower negative binding energy values are more successful. The interaction between ibuprofen and the serum albumin protein was used as a control group yielding a successful binding energy value of -2.004 kcal/mol.

The binding energy values between ABT 737 and both anti apoptotic proteins in humans and *planaria* resulted in values ranging from -0.7819 to 1.166 Kilocalories/mole. On the other hand, when ABT 263 was bound to proteins BCL-xL and BCL-2, in both *planarian* and humans, negative values were observed ranging from -9.468 kcal/mol to -11.01 kcal/mol.

The binding energy values observed in the interaction between ABT 263 and the antiapoptotic proteins in both species were considered successful energies because they yielded more negative values in kilocalories per mole.

Future Work

ABT 263 will be tested *in vivo* using whole cell proteomics and mass spectrometry to determine if Navitoclax can lower the concentration of BCL-2 and BCL-xL in *planarian* cells; thus, this method will be used to test ABT 263's potential to inhibit antiapoptotic proteins, BCL-xL and BCL-2, to induce apoptosis in a senescent *planarian* cell.