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VITAL ROLE OF AUTOPHAGY IN CANCER TREATMENT IN-SILICO STUDY

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Abstract-
Cancer is one of the leading deaths causing diseases all over world. Although anticancer therapies have been improved significantly, but it still has limited efficacy for tumor eradication and is highly toxic to healthy cells. That's why modulating autophagy for cancer treatment is an interesting or beneficial therapeutic approach. Autophagy is the process in which cell eats its own content. Autophagy is just like a self-healing process. Nutritional restriction or fasting is a promising custom to modulate autophagy and enhance the efficacy of anticancer therapies while protecting normal cells.
Going through the fasting body enter into the deep ketosis state, ketosis is a process that happens when body doesn't get enough amount of carbohydrate to burn for energy instead of glucose body burn fats for energy, where ketone body Beta hydroxybutyrate is synthesized by the body for providing energy.
Beta hydroxybutyrate binds to the AMPK to activate AMPK. AMPK binds with ULK1 for activation as ULK1 plays a core role in the activation of the autophagy pathway.
Keywords: Autophagy, Fasting, beta hydroxybutyrate, AMPK, ULK1

I. INTRODUCTION

Cancer is a leading cause of death worldwide, and its incidence is continually increasing. Although anticancer therapy has been improved significantly, but it still has limited efficacy for tumor eradication and is highly toxic to healthy cells. Thus, novel therapeutic strategies to improve chemotherapy, radiotherapy and targeted therapy are an important goal in cancer research. Macro autophagy is a conserved lysosomal degradation pathway for the intracellular recycling of macromolecules and clearance of damaged organelles and misfolded proteins to ensure cellular homeostasis [1]. That's why modulating autophagy for cancer treatment is an interesting or beneficial therapeutic approach. Nutritional restriction or fasting is a promising custom to modulate autophagy and enhance the efficacy of anticancer therapies while protecting normal cells. The 2016 Nobel Prize in Physiology or Medicine was awarded to Yoshinori Ohsumi for his initial elucidation of the morphological and molecular mechanisms of autophagy in the 1990s [2].

Autophagy is a cellular process for the degradation and elimination of misfolded proteins and damaged organelles that functions in adaptation to starvation, development, cell death and tumor suppression. It is the process in which the cell eats its own content. It is just like a self-healing. One of the important mechanisms of autophagy is an intracellular degradation pathway mediated by double membrane vesicles called autophagosomes. These autophagosomes deliver degraded cytoplasmic components to the lysosome to be recycled during stressful conditions. Autophagy responds to a range of cellular stresses, including nutrient deprivation, organelle damage, and abnormal protein accumulation. This autophagic process can be associated with cell death and cell survival. During nutrient deprivation, autophagy is enhanced to maintain a provision of important proteins and other nutrients to serve as an energy supply, thereby increasing cell survival [3].

Fasting boosts autophagy. Proper balanced nutritious fast (intake of fruits once in morning and staying hydrated by consuming water, lemon juice, or other fruit juices without any artificial ingredients at intervals) is important for taking body to keto stage. Staying hydrated is very essential as water is essential for the exfoliation of toxins. One should take care not to starve which enables formation of glycogen which is a body toxic. Fasting is a possible way to induce autophagy and promoting deep cleansing of body. Although fasting can help to detoxify the body. Under normal conditions, when the cell has sufficient nutrients, autophagy degrades damaged components in the cell (Charaka Samhita). Fasting

provokes the digestive cell to break the fat to glucose make a substance known as ketones to provide energy. This process is known as ketosis, where ketone body beta hydroxybutyrate is synthesized by the body for providing energy [4].

Beta hydroxybutyrate produced by fatty acid, activates AMPK (AMP- activated protein kinase) [5], beta hydroxybutyrate a ketone body that is used as an energy source in organs when blood glucose is low, oxidation in the liver under the fasting state [6].

Under glucose starvation, AMPK promotes autophagy by activating Ulk1 through phosphorylation [7].

Unc-51-like kinase 1 (ULK1) is a serine/threonine kinase that participates in the initiation of autophagy. Many studies have indicated that compounds that directly or indirectly target ULK1 could be used for tumor therapy [8].

In-Silico strategy has been used firstly performed protein-ligand docking then protein-protein docking and after then protein docking with metal ion. Molecular Docking is the process which predict the preferred orientation of one molecule to another molecule, when target bound with the ligand it forms a stable complex [9].

II. MATERIALS AND METHODS

1.1 Protein-Ligand Docking by using Auto Dock Vina tool

The publicly available target protein structures (database) are increasing rapidly that helps the identification of molecular targets. Target protein

Table1. Poses of beta hydroxybutyrate(ligand).

AMPK was selected from PDB (Protein Data Bank) then beta hydroxybutyrate a ligand molecule was selected from PubChem. Minimized the energy of AMPK by deleting water molecule, adding polar hydrogen and Kollman charges after then selected the docking site in the AMPK protein and carried out Protein- Ligand docking by using Auto Dock Vina which is a docking tool [10].

1.2 Protein-Protein Docking by using GRAMMAX

Target Protein namely, AMPK and ULK 1 was selected from PDB then carried out Protein-Protein Docking by using GRAMMAX, a docking web server. Visualized the docking result by using the JSmol.

1.3 Metal ion Docking with AMPK protein by using a MIB (Metal ion Binding)

Selected AMPK protein ID from the PDB and performed the protein docking with Ca2+.

1.4 ADME Test of Beta hydroxybutyrate by using Molinspiration

ADME (Absorption, Distribution, Metabolism, Excreation) test was performed to check ligand molecule beta hydroxybutyrate having a Drug like properties or not [11].

III. RESULT

Protein Ligand Docking:

Mode	Affinity (kcal/mol)	dist from rmsd l.b.	Best mode rmsd u.b.
1	-4.2	0.000	0.000
2	-4.1	1.644	2.202
3	-3.8	1.919	3.605
4	-3.8	2.039	2.588
5	-3.8	6.738	7.542
6	-3.8	10.144	11.426
7	-3.7	8.966	10.349
8	-3.6	18.885	19.194
9	-3.6	13.023	13.695

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Data presented in table 1 shows different poses of the ligand (beta hydroxybutyrate) with their energy. The pose of ligand with maximum score and least energy has been selected.
Beta hydroxybutyrate binds to AMPK at residues GLY-25, GLY-28 and ASP-157. As shown in the following Fig1.

Mode 1 ligand (beta hydroxybutyrate) pose has been selected as it having the least energy i.e. -4.2 kcal/mol).

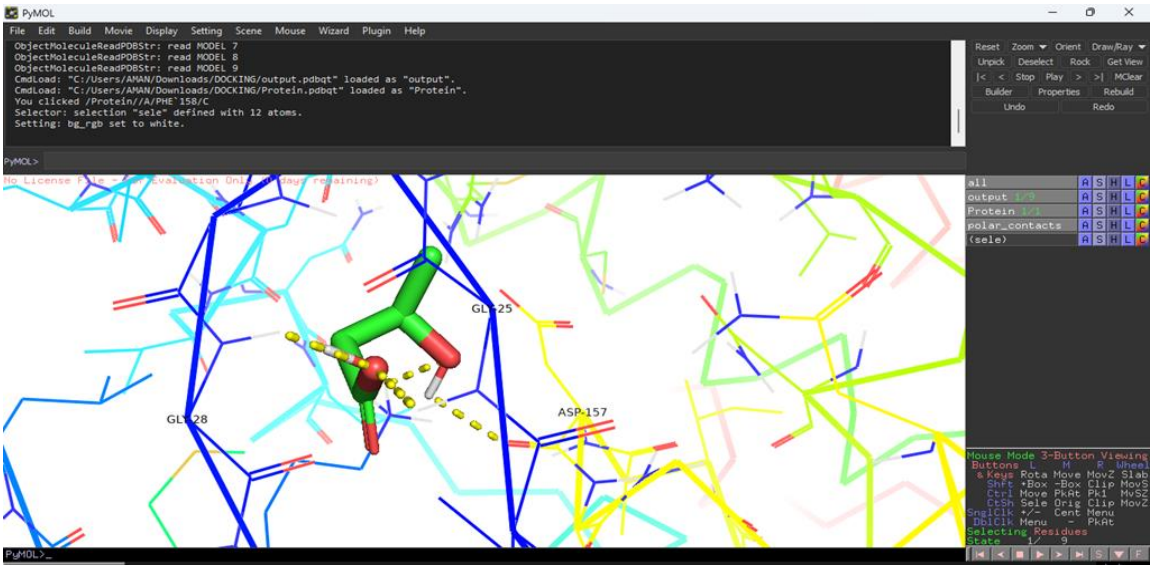

Fig1. Docking of beta hydroxybutyrate and AMPK

Protein-Protein Docking:

AMPK activating Ulk1 through binding at residues Asp 80 and Lys 255. As shown in the following Fig2.

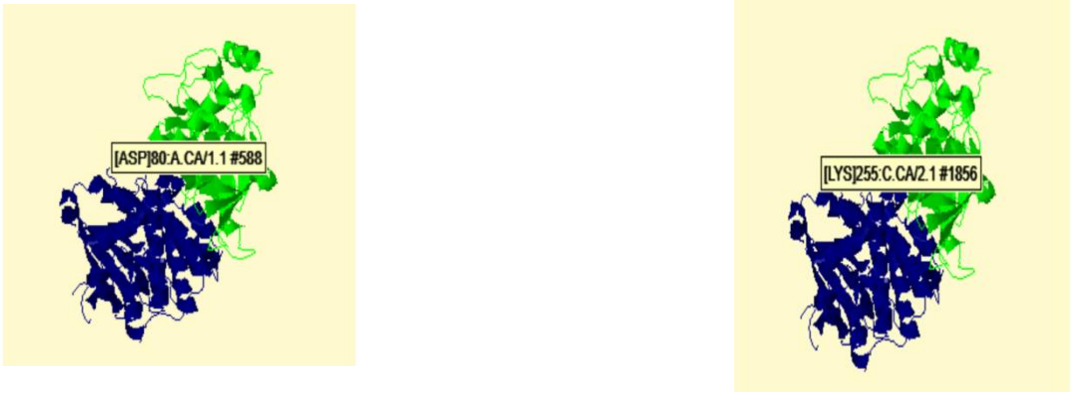

Fig2. Docking of AMPK and ULK1 protein

Metal ion Docking:

AMPK protein docking with metal ion Calcium. As shown in the following Fig3.

As shown in the Fig 3 Calcium ion having highest binding potential with AMPK protein

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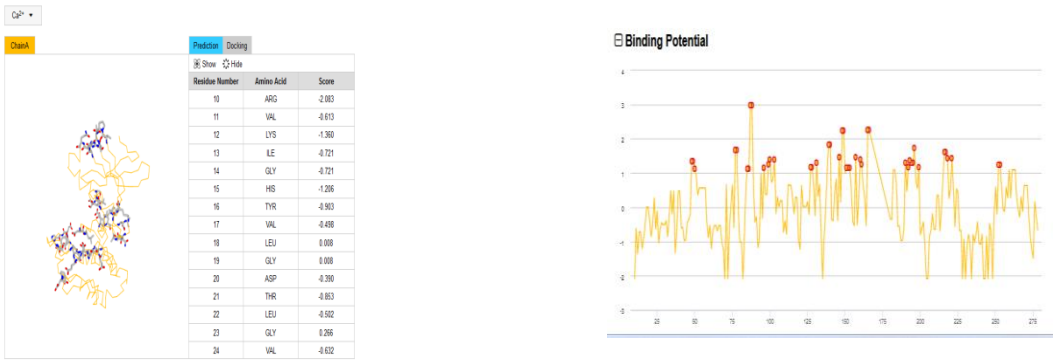

Fig 3. Docking of AMPK protein with calcium

ADME test of beta hydroxybutyrate:

Table 2: ADME test of BHB

Molecule name	miLogP	TPSA	natoms	MW	nON	nOHNH	nviolations	nrotb	volume
Beta Hydroxybutyrate	**-0.44**	**57.53**	**7**	**104.11**	**3**	**2**	**0**	**2**	**97.84**

As shown in the Table 2 Beta hydroxybutyrate having the drug like properties as its nviolations is 0.

IV. DISCUSSION

As per Charaka Samhita fasting triggers autophagy. During fast the ketone body releases Beta hydroxybutyrate. This should bind to AMPK to activate Ulk1 to trigger autophagy. As per the research by Deng Q et.al.,2015., also depicted in this work that Beta hydroxybutyrate successfully binds to AMPK protein. It is seen that Beta hydroxybutyrate successfully binds to AMPK protein at residues GLY-25, GLY-28 and ASP-157.

Further, Kim J et.al.,2011 stated that activated AMPK should trigger Ulk1 by binding to it to trigger autophagy which is depicted in our work that,

Beta hydroxybutyrate AMPK combination successfully binds with ULK1 confirming autophagy by fasting. The Beta hydroxybutyrate-AMPK combination binds with ULK1 binding at residues Asp 80 and Lys 255 confirming research by Kim J et.al.,2011.

Further, in-vitro receptor ligand binding studies taking compound Beta hydroxybutyrate with protein AMPK can be done to justify the activation of AMPK. Further, binding studies with activated AMPK with Ulk1 for triggering Ulk1 can be done to justify autophagy

V. CONCLUSION

It is seen that Beta hydroxybutyrate successfully binds to AMPK protein which confirms that it activates AMPK as per research by Deng Q et.al.,2015. This combination binds with ULK1 confirming research by Kim J et.al.,2011. Beta hydroxybutyrate additional with Calcium ion enhance the efficiency of AMPK protein.

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