TARGETING HYPOXIA INDUCIBLE FACTORS WITH NATURAL PHYTOCHEMICALS – A BIOINFORMATICS APPROACH

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Abstract

HIF-1α (hypoxia inducible factor 1 alpha) is a protein that controls cell growth and differentiation and has been linked to cancer in humans. In cancer cells, HIF-1 triggers the carcinogenesis cascade function. The HIF-1 receptor is thought to be the initiator of signaling and its `over expression has been linked to a variety of human cancers, including breast cancer, lung cancer, and colon cancer. As a result, HIF-1 becomes a possible therapeutic target in the development of HIF-1 inhibitors. The aim of this study is to look into natural potential inhibitors. To accomplish this, the chemical structures of all substances were downloaded from the PubChem database. PyRx was used to conduct the docking and Pymol was used to evaluate the resulting binding modes. Berberine, a natural compound demonstrated the strongest binding modes as compared to other inhibitors, with the lowest binding energies (-8.6 kcal/mol). This study indicates that Berberine interacts favorably with the necessary amino acid residues at HIF-1's catalytic site. Present findings of the molecular docking analysis may be useful as a compound model for further in vitro and in vivo research.

Keywords: HIF-1α, Berberine, Breast Cancer, PyRx, Molecular docking

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1. Introduction

For decades, hypoxia has been extensively studied within the tumor microenvironment, where it has a variety of effects on malignant cells (1, 2). Although the O_2 supply in normal tissues meets the requirement for cell growth, the diffusion of O2 from a blood capillary into a tumor mass of 1-2 mm^3 is limited due to structural and functional defects in the newly developed tumor vessels, resulting in the formation of hypoxic area within the tumor mass (3).

Breast cancer is the most commonly diagnosed cancer in women worldwide and a leading cause of death (4, 5). About 25%–40% of invasive breast cancers have hypoxic areas (6). The median partial pressure of oxygen (pO2) in normal human breast tissue has been determined to be 65 mmHg (1 mmHg=133.3 Pa) by polarographic electrode studies. Human breast cancers, on the other hand, have a median pO2 of 10 mmHg. Furthermore, over half of all breast cancers surveyed had a pO2 value of less than 2.5 mmHg (7, 8). Intra-tumoral hypoxia has been shown to have a detrimental effect on breast cancer patient survival, regardless of prognostic factors such as clinical tumor level, histological grade, and lymph node status. Hypoxic tumors have a more aggressive phenotype, an increased risk of metastasis, increased resistance to radiotherapy and chemotherapy, and induced immune suppression in cancer (9, 10).

At diagnosis, a high level of HIF-1 predicts early relapse and metastasis and is associated with lower clinical outcomes in human breast cancer (11, 12). It has been documented that the triple-negative breast cancer subgroup expresses more HIF-1 target genes (Cancer Genome Atlas Network, 2012). HIF-1 is needed for a variety of critical aspects of breast cancer biology, including angiogenesis; stem cell maintenance, metabolic reprogramming, EMT, invasion, metastasis, and resistance to radiation and chemotherapy (13). By mediating hypoxia-induced expression of mRNA-encoding genes, HIF-1 plays a critical role in breast cancer metastasis. HIF-1 also controls non-coding RNA expression, which plays a critical role in migration, invasion, and metastasis. HIF-1 could be a priority for the development of new lead compounds for breast cancer.

For several years, natural bioactive compounds extracted from plants have been used to preserve health and as remedies (14-20). In the pharmaceutical sector, phytochemical constituents of plants have been a crucial pipeline for the discovery of bioactive substances (21-23). The present research examined the anticancer activity of many natural bioactive compounds using a molecular docking method.

2. Materials and methods

Protein structure preparation

The three dimensional structure of HIF-1α (Entry PDB code: 1H2K) was retrieved from RSCB Protein Database. The attached ligand in the protein structure was removed from the binding site and saved to a new file format: pdbqt. The Gasteiger charges and the solvation conditions were added to the protein structure using the AutoDockTool.

Ligand Preparation

The natural compounds (Berberine, curcumin, genistein, quercetin and resveratrol) have been obtained from the Pubchem database in SDF format. And then all the compounds were converted to .PDB format using Online Smiles Translator. The charges were further repaired by inserting partial gasteiger charges and then push the autodock. Then the structure of the compounds was opened on PyRx by clicking on Load Molecule and making ligand.

Molecular docking

AutoDock (V. 4.0) was used in the PyRx Interface to validate the binding capabilities of the interactions between selected compounds and HIF-1α (24, 25) (Trott *et al.,* 2010; Morris *et al.,* 2009). During the docking phase, ligand was thought to be flexible and the protein was considered to be rigid. The grid configuration file was generated by using the Pyrex Auto Grid engine. The implementation was also used to know / predict the amino acids that come in contact with ligands at the active protein site. Values less than 1.0Å in the position root-mean-quarter deviation (RMSD) were considered to be optimal and grouped together to find an acceptable binding. The highest binding energy (most negative) was found to be a ligand with high binding affinity.

3. Results and discussion

PyRx was used for the molecular docking studies. The docked conformation corresponding to the lowest binding energy was selected as the most probable binding conformation. The binding score and hydrogen bond details of selected five compounds were shown in Table 1. Which all the ligands were embedded within the active site of target protein were observed forming hydrogen bonds in the active site of target protein. All the five compounds showed very good binding with HIF-1α with low binding score range from -8.6 kcal/mol to -7.6 kcal/mol. Hydrogen bond interaction also indicates that all the compounds found effectively in the active site of HIF-1α.

Table 1: Molecular docking results obtained from PyRx

S.no	Compound name	Docking score	Hydrogen bond interaction	Distance A◦
1	Berberine	-8.6	GLN-147 GLN-203	2.2 2.7
2	Curcumin	-8.2	GLU-202 GLN-239	1.5 2.4
3	Genistein	-7.8	GLN-147 ASP-201 ARG-238 GLN-239	2.7 2.4 2.0 2.2
4	Quercetin	-8	SER-91 THR-149 ASP-201 ARG-238	2.2 2.2 2.5. 2.3
5	Resveratrol	-7.6	ARG-238 GLN-239	1.4 2.2

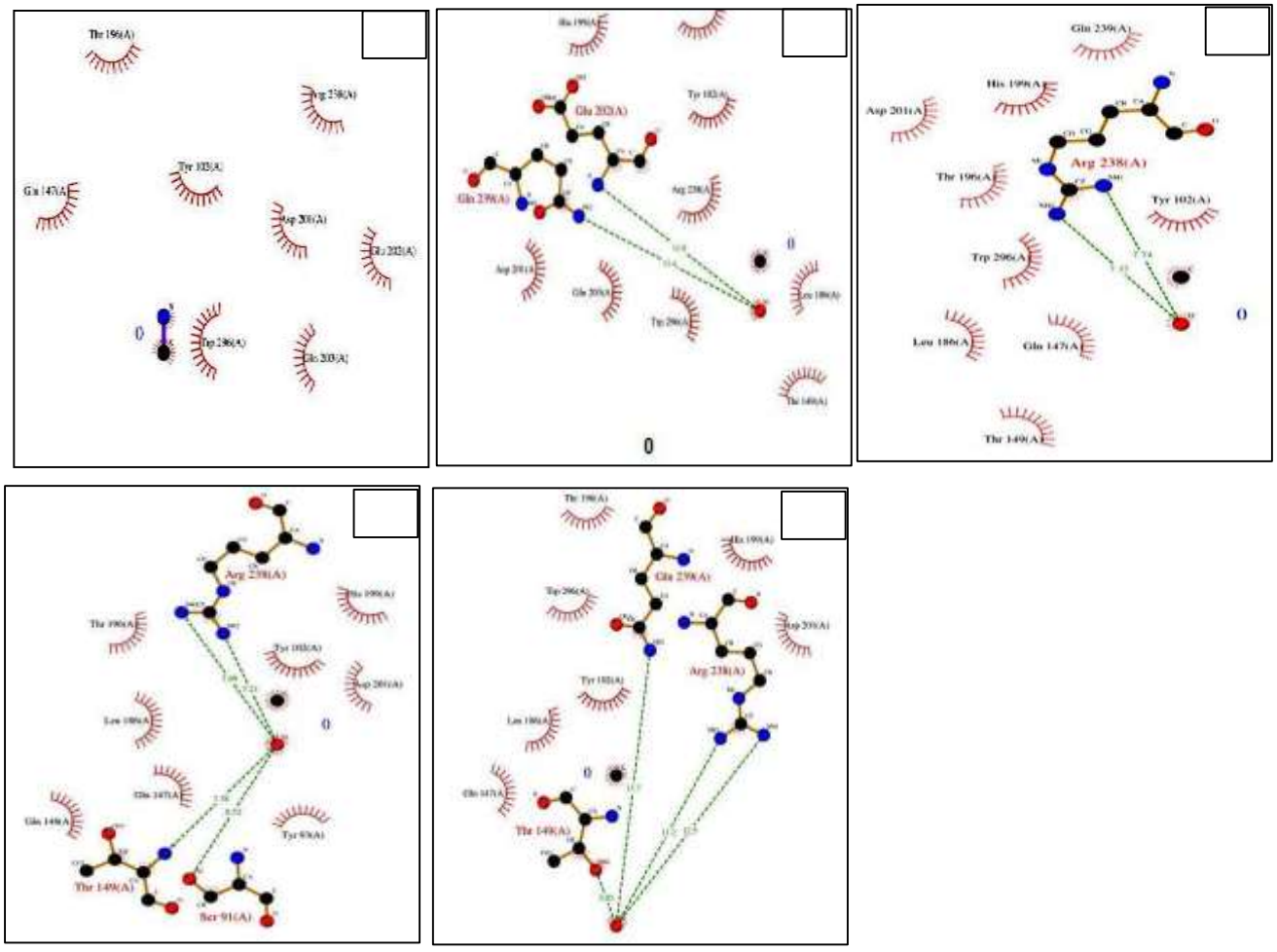

Figure 1: Molecular interaction of HIF-1α with Berberine (A), Curcumin (B), Genistein (C), Quercetin (D) and Resveratrol (E).

All the five compounds showed good number of hydrogen bonds with HIF-1α. Among the five compounds berberine showed the lowest finding score of -8.6 kcal/mol and it's formed the two hydrogen bond interactions with GLN-147 and GLN-203. The distance of the hydrogen bond interaction also occurs within 3A°. Hence, this also indicates that all compounds showed good binding pattern with HIF-1α. The molecular interactions of all the five compounds with HIF-1α were shown in figure 1A-E.

4. Conclusion

Medicinal plants play important roles in the development of modern therapeutic agents. This study conclusively demonstrated that natural compounds may acts as good source for potential therapeutics. On the basis of our results, it can be concluded that the berberine was powerful phytocompound, which offers protective effect against cancer. Thus, berberine was selected to be the best lead as anticancer agent in silico based on the lowest binding score and hydrogen bond interaction These *in silico* results could be beneficial as a compound model for further experimentally *in-vitro* and *in-vivo* assays to elucidate the exact mechanism of inhibitory activity and to examine its potential therapeutic effects.

Conflict of interest

There is no conflict of interest from any of the authors.

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Figure Caption

Molecular interaction of HIF-1α with a) Berberine b) Curcumin c) Genistein d) Quercetin e) Resveratrol