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Natural compounds as inhibitors of Beta catenin: An *in silico* study

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Abstract

Demand for a new anti-cancer drug has been dramatically increasing in the recent years. The Wnt/β catenin pathway acts as significant modulator of colon cancer and now serve as a new drug target for the development of anti-cancer/anti-tumour molecules. In the present study, five compounds namely apigenin, galangin, luteolin, proanthocyanidin and silibinin were evaluated on their docking behaviour on beta (β) catenin protein. The docking studies and atomic contact energy (ACE) calculations shown that galangin exhibited the highest binding energy (-194.11 kcal/mol). Interestingly two ligands namely apigenin and galangin interacted with Ser246 amino acid residue of β catenin. Thus, the results of this present study exhibited the potential of these five ligands as β catenin inhibitory agent and also as anti-cancer agents.

Keywords: Beta catenin, Apigenin, Galangin;Luteolin; Proanthocyanidin; Silibinin.

I. Introduction

In the developing world the colorectal cancer [colon and rectal] has become the third most common cancer among men and women[1,2]. The initiating and driving factor of majority colorectal cancers (CRC) demonstrate the uncontrolled activation of the WNT signaling pathway, the WNT signaling is mediated by β-catenin gene [3]. The β-catenin is a protein which acts as a transcription factor, which is also involved in other types of cancers such as hepatocellular cancer, malignant breast cancer and endometrial cancer [4]. The glycolipoproteins secreted in the WNT pathway is important mechanism that directs cellular proliferation, cell polarity and cell fate determination during embryonic developmental stage and tissue homeostasis[5]. The WNT signaling, functions by regulating the amount of the transcriptional co-activator β-catenin that controls the gene expression functions[6, 7].Numerous phytoconstituents are potent in the treating several cancer types including colorectal cancers. Previously, we reported that phytoconstituents having potent anticancer activity by modulating several cellular signalling pathways [8-10]. In the present study, we selected five compounds (namely apigenin, galangin, luteolin, pro-anthocyanidin and silibinin) and were evaluated on their docking behaviour on β-catenin protein.And hereby the results of the present study will give new insight for the future design these anti-cancer agents.

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II. Materials and methods

Ligand preparation

Chemical structures of the ligands namely i) apigenin (CID5280443); ii) galangin (CID5281616); iii) luteolin (CID5280445); iv) proanthocyanidin (CID108065) and v) silibinin (CID31553) were retrieved from PubMed (www. pubmed.com).Then the ligands drawn in ChemBio Draw Ultra 12.0 and the molecular mechanics (MM2) energy minimization of ligands was carried out by ChemBio 3D Ultra 12.0, according to the reported procedure [11] (Vijayakumar et al., 2017). These minimized energy structures were used for further Patch Dock study.

Target protein identification and preparation

The three-dimensional (3D) structure of the target proteinBeta (β) catenin (PDB 1JDH with resolution of 1.9 A^0 were obtained from the Research Collaborator for Structural Bioinformatics (RCSB) Protein Data Bank (www.rcsb.org) A chain of this protein was pre-processed by removing another chain (B), ligands, in addition to the crystallographically observed water particles (water without hydrogen bonds). UCSF Chimera software (www.cgi.ucsf.edu/chimera) was used to prepare the above-mentionedprotein (Radhakrishnan et al., 2017).

Docking studies

Docking studies were carried out by the PatchDock online server (http://bioinfo3d.cs.tau.ac.il/PatchDock). PatchDock adopts geometry-based molecular docking algorithm method was used to recognize the binding scores, by binding residues atomic contact energy of the given ligands. The docking results were obtained through the email address. We also use to get uniform resource locator (URL) which provides the top 20 solutions in a table form via email. From these, the top one solution (the docked protein-ligand complex) was selected and downloaded in a database (pdb) file format (Radhakrishnan et al., 2017). Further, the binding site analysis was carried out by PyMOL software (www.pymol.org).

III. Results and discussion

The over activation of WNT pathway leads to colorectal cancer,it may cause by either Adenomatous polyposis coli (APC)or β-catenin gene mutation[**13**]. APC forms complex in the cytosol with β-catenin, Glycogen synthase kinase (GSK) -3β, Axin and casein kinase 1. When mutation occurs in APC that results the movement of free β-catenin into nucleus. Non-p-β-catenin will bind to Leukocyte Erythroid factor (LEF)-1 and T-cell factor (TCF), activates the cell proliferation genes such as C-myc and Cyclin D1 [**13, 14**]. About 80% of colorectal cancer (CRC) has shown dysregulation of β-catenin in WNT signalling pathway[**14**]. Thus,β-catenin protein, now serve as new drug target for colorectal cancer.

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Several secondary metabolites from naturally sources have been reported to inhibit or suppress the β catenin signaling by five different molecular mechanisms such as I) Down-regulating β catenin expression at protein and/ or mRNA levels respectively; II) Modulating β catenin phosphorylation and inducing its inactivation; III) Promoting β catenin protein ubiquitination and proteasomal degradation; IV) Inhibiting β catenin nuclear translocation, and V) Other [**15**]. The docking studies and atomic contact energy (ACE) calculations shown that galangin exhibited the highest binding energy (-194.11 kcal/mol). In contrast proanthocyanidin and silibinin showed very least binding energy (+100.77 and +55.90 kcal/mol respectively) with beta catenin (as shown in the table 1), which might due to unfavourable interactions phenomenon as reported by Castro and co-workers [**16**].Interestingly two ligands namely apigenin and galangin interacted with Ser246 amino acid residue of β catenin, the present study was in par with the earlier reports [**17-20**]. Similarly, Ashokkumar and Sudhandiran [**21**] reported that luteolin inhibits colon carcinogenesis through Wnt/ β catenin signalling pathway. In the present study, luteolin does not interact with aminoacid residue of β catenin, while shown to dock with β catenin(as shown in the table 1 and figure 1).

IV. Conclusion

In the present study, two ligands namely proanthocyanidin and silibinin exhibited weak or poor binding energy with that of β catenin. Thus, the results of this present study exhibited the potential of these five ligands as β catenin inhibitory agent and also as anti-cancer agents.

Table 1: Binding energy analysis of five ligands with that of β catenin protein using PatchDock

S,no	Ligand	-ACE* (kcal/mol)	Interactions of amino acid residues	Bond distance (A°)
1.	Apigenin	171.14	Gln203	3.3
			Lys242	2.8
			Ser246	3.5
2.	Galangin	194.11	Ser246	2.1
3.	Luteolin	190.79	No interactions	-
4.	Proanthocyanidin	+100.77	Asn430	1.8 and 3.5
			Lys435	2.6
			Arg515	3.3
5.	Silibinin	+55.90	Arg515	2.0 and 2.9
			Gly572	2.7

**Note: ***ACE-Atomic contact energy

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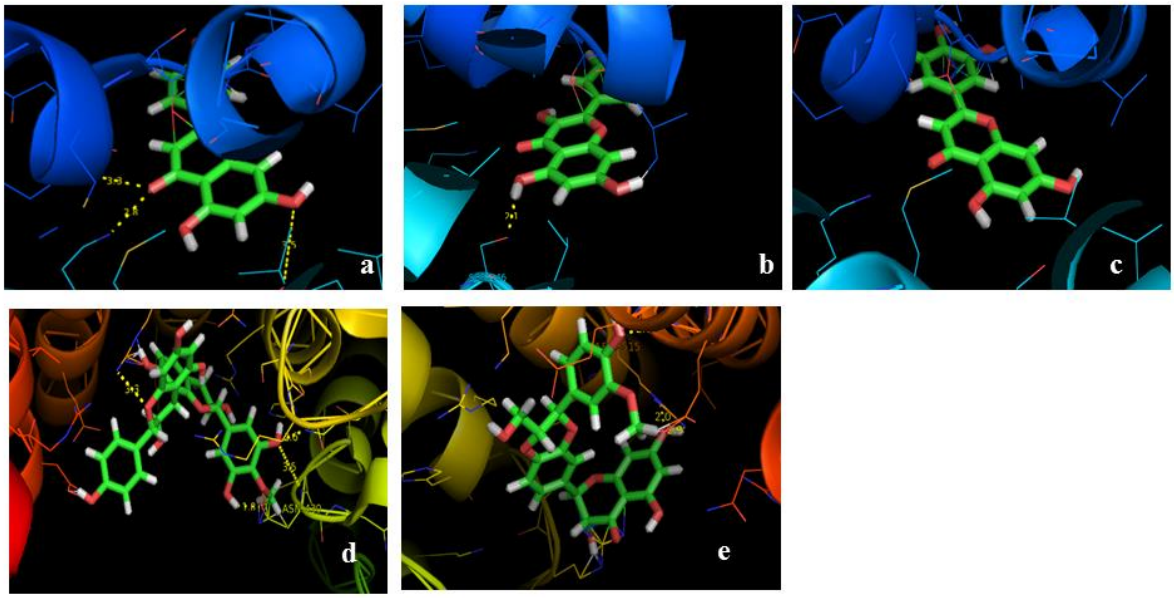
Figure 1 represents the five ligands [a) Apigenin, b) Galangin, c) Luteolin, d) Proanthocyanidin and e) Silibinin]have shown to dock with β catenin



References

1. Miyamoto Y.; Hiyoshi Y.; Sawayama H.; Tokunaga R.; Baba H. Precision medicine for adjuvant chemotherapy of resected colorectal cancer. Ann Gastroenterol Surg, **2020,**4(6), 635-645. doi: 10.1002/ags3.12397.
2. Jin L.; Kim E.Y.; Chung T.W.; Han C.W.; Park S.Y.; Han, J.H.; Bae, S.J.; Lee, J.R.; Kim, Y.W.; Jang, S.B.; Ha K.T. Hemistepsin A suppresses colorectal cancer growth through inhibiting pyruvate dehydrogenase kinase activity. Sci Rep. **2020**, 10(1), 21940. doi: 10.1038/s41598-020-79019-1.
3. Kasprzak A. Angiogenesis-Related Functions of Wnt Signaling in Colorectal Carcinogenesis. Cancers (Basel). **2020**, 12(12), E3601. doi: 10.3390/cancers12123601.
4. Harmston N, Lim JYS, Arqués O, Palmer HG, Petretto E, Virshup DM, Madan B. Widespread repression of gene expression in cancer by a Wnt/β-catenin/MAPK pathway. Cancer Res. 2020, canres.2129.2020. doi: 10.1158/0008-5472.CAN-20-2129.
5. Tang Q, Chen J, Di Z, Yuan W, Zhou Z, Liu Z, Han S, Liu Y, Ying G, Shu X, Di M. TM4SF1 promotes EMT and cancer stemness via the Wnt/β-catenin/SOX2 pathway

in colorectal cancer. J Exp Clin Cancer Res. 2020 Nov 5;39(1):232. doi: 10.1186/s13046-020-01690-z.

6. Koni M, Pinnarò V, Brizzi MF. The Wnt Signalling Pathway: A Tailored Target in Cancer. Int J Mol Sci, **2020**, 21(20):7697. doi: 10.3390/ijms21207697.
7. Stefanski CD, Prosperi JR. Wnt-Independent and Wnt-Dependent Effects of APC Loss on the Chemotherapeutic Response. Int J Mol Sci, **2020**, 21(21), 7844. doi: 10.3390/ijms21217844.
8. Radhakrishnan Narayanaswamyand Mallappa Kumara Swamy (2018). Chapter Elucidation of Mechanisms of Anticancer Plant Compounds Against the Tumor Cells. In: Mohd Sayeed Akhtar and Mallappa Kumara Swamy (Eds.)*Volume 4.Anticancer Plants: Mechanisms and Molecular Interactions*, page no: 99-130, Springer Nature Singapore (Springer Publisher).
9. Mohebali N.; Pandurangan A.K.; Mustafa, M.R.; Anandasadagopan, S.K.; Alagumuthu T. Vernodalin induces apoptosis through the activation of ROS/JNK pathway in human colon cancer cells. J Biochem Mol Toxicol, **2020**, 34(12), e22587. doi: 10.1002/jbt.22587.
10. Pandurangan A.K.; Ismail S.; Esa N.M.; Munusamy M.A. Inositol-6 phosphate inhibits the mTOR pathway and induces autophagy-mediated death in HT-29 colon cancer cells. Arch Med Sci. **2018**, 14(6), 1281-1288. doi: 10.5114/aoms.2018.76935.
11. Vijayakumar V.; Radhakrishnan N.; Rameshkumar C. Molecular docking analysis of imidazole derivatives and polybenzimidazole analogues as inhibitors of superoxide dismutase (SOD) and Xanthine oxidase (XO). IEEE International Conference on Smart Technologies and Management for Computing, Communication, Controls, Energy and Materials (ICSTM),**2017**, 513-516. DOI: 10.1109/ICSTM.2017.8089213.
12. Radhakrishnan N. Molecular docking analysis of Alginate oligosaccharides (Alg2-Alg6) as bacterial collagenase inhibitor. J. Appl. Cosmetol. **2017**, 35, 15-23.
13. Bian J.; Dannappel M.; Wan C.; Firestein R. Transcriptional Regulation of Wnt/β-Catenin Pathway in Colorectal Cancer. Cells, **2020**, 9(9), 2125. doi: 10.3390/cells9092125.
14. Nie X.; Liu H.; Liu L.; Wang Y.D.; Chen W.D. Emerging Roles of Wnt Ligands in Human Colorectal Cancer. Front Oncol. **2020**, 10, 1341. doi: 10.3389/fonc.2020.01341.
15. Yu W.K.; Xu, Z.Y.; Yuan, L.; Mo, S; Xu, B.; Cheng, X.D.; Qin, J.J. Targeting β-Catenin Signaling by Natural Products for Cancer Prevention and Therapy. Front Pharmacol. **2020**, 11, 984. doi: 10.3389/fphar.2020.00984.
16. Castro J.S.; Trzaskowski B.; Deymier P.A.; Bucay J.; Adamowicz; L.; Hoying; JB. Binding affinity of fluorochromes and fluorescent proteins to Taxol™ crystals. Mat Sci Eng: C, **2009**, 29(5), 1609-1615.
17. Shukla, S.; MacLennan, G.T.; Flask, C.A.; Fu, P.; Mishra, A.; Resnick, M.I.; Gupta, S. Blockade of β-catenin signaling by plant flavonoid apigenin suppresses prostate carcinogenesis in TRAMP mice. Cancer Res, **2007**, 67(14), 6925-6935. DOI: 10.1158/0008-5472.CAN-07-0717

18. Xu M.; Wang S.; Song Y.U.; Yao J.; Huang K.; Zhu X. Apigenin suppresses colorectal cancer cell proliferation, migration and invasion via inhibition of the Wnt/β-catenin signaling pathway. Oncol Lett, **2016**, 11(5), 3075-3080. DOI: 10.3892/ol.2016.4331.
19. Gwak J.; Oh J.; Cho M.; Bae S.K.; Song I.S.; Liu K.H.; Jeong Y.; Kim D.E.; Chung Y.H.; Oh, S. Galangin suppresses the proliferation of β-catenin response transcription-positive cancer cells by promoting adenomatous polyposis coli/Axin/glycogen synthase kinase-3β-independent β-catenin degradation. Mol pharmacol, **2011**, 79(6), 1014-1022. DOI: 10.1124/mol.110.069591.
20. Sferrazza G.; Corti M.; Brusotti G.; Pierimarchi P.; Temporini C.; Serafino, A.; Calleri E. Nature-derived compounds modulating Wnt/β-catenin pathway: a preventive and therapeutic opportunity in neoplastic diseases. Acta Pharmaceutica Sinica B, **2020**, 10(10), 1814-1834. DOI: 10.1016/j.apsb.2019.12.019.
21. Ashokkumar P.; Sudhandiran G. Luteolin inhibits cell proliferation during zoxymethane-induced experimental colon carcinogenesis via Wnt/ β-catenin pathway. Invest New Drugs. 2011, 29(2), 273-84. doi: 10.1007/s10637-009-9359-9.
22. Differential expression of Helios, Neuropilin-1 and FoxP3 in head and neck squamous cell carcinoma (HNSCC) patients A.A.Mohamed Adil, Anil Kumar Bommanabonia, Anandraj Vaithy, Sateesh Kumar 3biotech 9 (178)
23. Protagonist of Immuno-Profiling, Immuno-Scoring, and Immunotherapy Towards Colitis-Associated Cancer: Systematic Review, Mohamed Adil a.a, AK Pandurangan, M Waseem, N Ahmed Diagnostic and Treatment Methods for Ulcerative Colitis and Colitis 2020
24. Emerging Role of Mitophagy in Inflammatory Diseases: Cellular and Molecular Episodes, Mohamed Adil AA, S Ameenudeen, A Kumar, S Hemalatha, N Ahmed, N Ali 2020 Curr Pharm Des. 2020;26(4):485-491. doi: 10.2174/1381612826666200107144810
25. Increased Expression of TGF-β and IFN-γ in Peripheral Blood Mononuclear Cells (PBMCs) Cultured in Conditioned Medium (CM) of K562 Cell Culture AAM Adil, L Vallinayagam, K Chitra, S Jamal, AK Pandurangan, N Ahmed Journal of Environmental Pathology, Toxicology and Oncology 38 (2)
26. Cancer immunotherapy: Targeting immunosuppressive tumor microenvironment NA A.A Mohamed Adil Oncobiology and Targets 2014