Computational analysis of Murine Double Minute 2 and UBS109 in pancreatic cancer

Veda Muppala, Santosh Behera, Basal El-Reyes and Ganji Purnachandra Nagaraju

[1]*Department of Biotechnology, National Institute of Pharmaceutical Education and Research, (NIPER), Ahmedabad, Gujarat, India*

[2]*Department of Hematology and Oncology, Heersink School of Medicine, University of Alabama, Birmingham, AL 35233, USA.*

BACKGROUND & OBJECTIVE

Overexpression of Murine Double Minute 2 (MDM2) associated with the inhibition of tumor suppressors, enhanced epithelial-mesenchymal transition (EMT), and upregulation of oncoproteins in pancreatic cancer (PC). Curcumin is an extensively studied molecule due to its various anticancer properties. Curcumin is known to upregulate p53, p21, and p27. At the same time, downregulate MDM2, cyclin D1, and cyclin E. UBS109 is a mono-carbonyl analog of curcumin that exhibits comparable anti-tumor and anti-inflammatory characteristics without the restrictions of curcumin. This investigation intends to target MDM2 by curcumin and UBS109 in PC.

MATERIALS & METHODS

The sequence and structural information of Murine Double minute 2 (MDM2) were retrieved from UniProt ID Q00987 (MDM2_HUMAN) and PBD ID 6Q9L. The structural information of curcumin and its analog UBS109 was obtained from PubChem with compound IDs 969516 and 1536727. AutoDock 4.2 was used for molecular docking studies. The GROMACS (version 2019.4) molecular dynamics (MD) simulation package was used to analyze the apo (MDM2 protein only) and holo (MDM2-curcumin complex: holo1; and MDM2-UBS109 complex: holo2) states. All 2D plots were created using Graphing, Advanced Computation, and Exploration 5.1.23 for data analysis of MD simulations. Pancreatic cancer cell lines (As PC-1, and PANC-1) were used. Protein expression was analyzed using a western blot.

OBSERVATIONS

Table I. Docking scores of Curcumin and UBS109 with MDM2

Target	PubChem CID	Drug	Binding Energy (kcal/Mol)	Ligand Efficiency	Inhibition Constant (μm)	No. of H - Bonds	H-Bond Forming Residues	Average Distance Of H-Bonds (Å)
MDM2 (PDB ID: 6Q9L)	969516	Curcumin	-6.67	-0.25	12.84	1	LYS51	3.044
MDM2 (PDB ID: 6Q9L)	1536727 Analog (UBS109)	UBS109	-6.60	-0.3	14.52	0	0	0

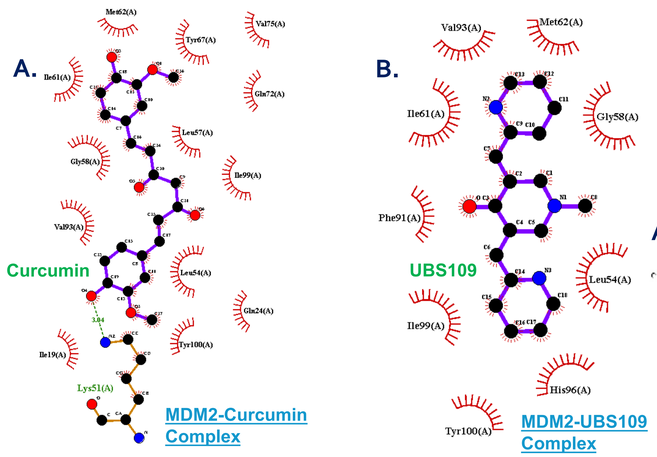

*Fig.1. Intermolecular H-bonds, electrostatic and hydrophobic interactions established amid **(A)** MDM2 -Curcumin **(B)** MDM2-UBS109. Images generated via LigPlot+ tool.*

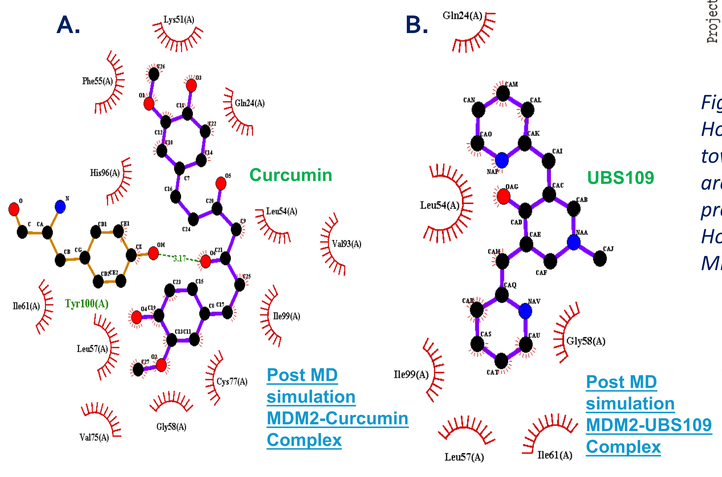

*Fig.2. Deviation of H-bonds during 50ns simulation in MDM2-Curcumin and MDM2-UBS109 **(A)** Post-MD simulations intermolecular hydrogen bond, electrostatic interaction and hydrophobic interaction formed between MDM2-Curcumin **(B)** Post-MD simulations intermolecular H-bond, electrostatic interaction and hydrophobic interaction formed between MDM2-UBS109. Images generated via LigPlot+ tool.*

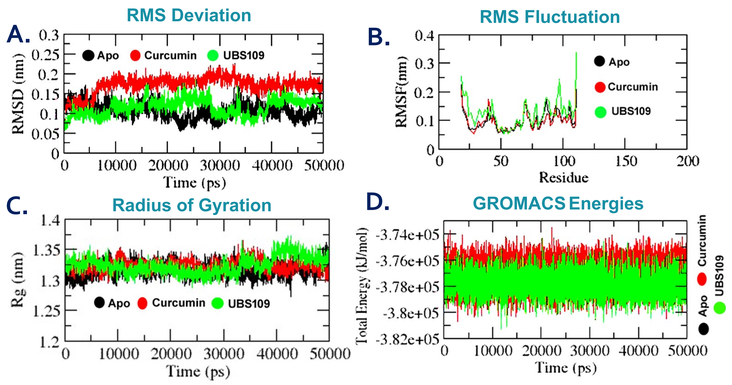

*Fig. 3. Conformational stability of Apo and Holo 1, Holo 2 during 50ns (nanoseconds) of MD simulations **(A)** Backbone-RMSD of MDM2. **(B)** Cα-RMSF profile of MDM2 **(C)** Radius of gyration (Rg) profile of MDM2 **(D)** Total energy of Apo (black), Holo1 (red) and Holo2 (green) states during 50ns of MD simulations. RMSD, root mean square deviation; RMSF, root mean square fluctuation; MD, molecular dynamics.*

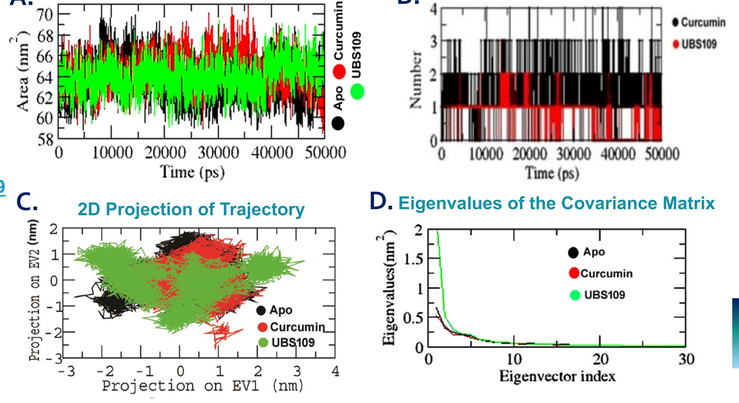

*Fig. 4. **(A)** Solvent accessible surface analysis (SASA) of Apo, Holo 1, and Holo 2 during 50ns of MD simulations. **(B)** Deviation of H-bonds contributed towards the interaction during 50 nsec simulation in Holo1 and Holo2 states are displayed by black and red lines, respectively. **(C)** Cloud represents the projection of trajectories eigenvectors (EV1 and EV2) (Black: Apo; Red: Holo1; Green: Holo2). **(D)** Projection of the motion of Apo and Holo states of MDM2 in phase space along the first two principal eigenvectors.*

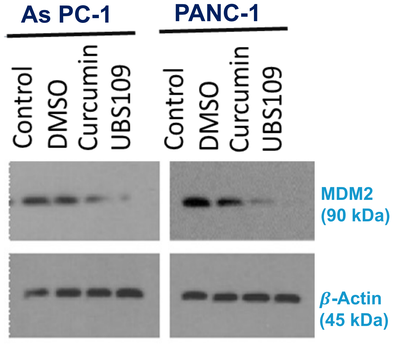

Fig. 5. Treatment with curcumin (25μM) and its analog UBS109 (2μM) to examine MDM2 levels in AsPC-1 and PANC-1 cell lines. Cells were harvested post treatment with control, curcumin, or UBS109. Protein were extracted and assessed using Western Blot as defined within Materials and Methods section.

RESULTS

- Using computed atlas surface topography of proteins (CASTp) we identified active sites residues (Ile92, Gln24, Lys51, Leu54, Phe55, Leu57, Gly58, Ile61, Met62, Tyr67, Gln72, Val93, His96, Ile99, Tyr100) for binding of the ligand.
- Using AutoDock 4.2, we found the interaction between MDM2-curcumin and MDM2-UBS109. Post-MD simulations revealed that UBS109 exhibited higher reactivity and higher kinetic stability than curcumin (GROMACS software).
- Treatment with curcumin or UBS109 caused a low MDM2 protein level in AsPC-1 and PANC-1 cells compared to treatment with 1% DMSO. β-Actin is used as a loading control.
- The results revealed a slightly higher binding affinity for MDM2-curcumin (-6.67) as compared to MDM2-UBS109 (-6.60) complex. One H-bond was observed in MDM2-curcumin complex formed by LYS51. Lower Hydrophobic interactions were observed in MDM2-UBS109.

CONCLUSIONS & FUTURE DIRECTIONS

- The collective outcomes of in silico and in vitro analyses revealed that UBS109 can efficiently bind to MDM2, decreasing MDM2 expression.
- Moreover, MD simulations reveal that UBS109 binding with MDM2 leads to decreased mobility of the residues, suggesting that UBS109 stabilizes MDM2.
- In the docking results, one of the hydrogen bonds between MDM2 and Curcumin was at LYS51 (Lysine) which later compensated with residue TYR100 (Tyrosine) during post MD simulations. This indicates that there is a rotation of Curcumin within the active sites of the MDM2.
- UBS109 had 9 hydrophobic interactions but later showed 6 hydrophobic interactions. This shows through the Post MD simulations results that there is an increase in other type of interactions and forces (strong electrostatic forces, etc.)

REFRENCES

[1] Díaz Osterman CJ, Gonda A, Stiff T, Sigaran U, Valenzuela MM, Ferguson Bennit HR, Moyron RB, Khan S, Wall NR. Curcumin Induces Pancreatic Adenocarcinoma Cell Death Via Reduction of the Inhibitors of Apoptosis. Pancreas. 2016 Jan;45(1):101-9. doi: 10.1097/MPA.0000000000000411. PMID: 26348467.

[2] Nagaraju GP, Zhu S, Wen J, Farris AB, Adsay VN, Diaz R, Snyder JP, Mamoru S, El-Rayes BF. Novel synthetic curcumin analogues EF31 and UBS109 are potent DNA hypomethylating agents in pancreatic cancer. Cancer Lett. 2013 Dec 1;341(2):195-203. doi: 10.1016/j.canlet.2013.08.002. Epub 2013 Aug 7. PMID: 23933177.

[3] Nagaraju GP, Zhu S, Ko JE, Ashritha N, Kandimalla R, Snyder JP, Shoji M, El-Rayes BF. Antiangiogenic effects of a novel synthetic curcumin analogue in pancreatic cancer. Cancer Lett. 2015 Feb 28;357(2):557-65. doi: 10.1016/j.canlet.2014.12.007. Epub 2014 Dec 9. PMID: 25497868.

[4]. Bimonte S, Barbieri A, Leongito M, Piccirillo M, Giudice A, Pivonello C, de Angelis C, Granata V, Palaia R, Izzo F. Curcumin AntiCancer Studies in Pancreatic Cancer. Nutrients. 2016 Jul 16;8(7):433. doi: 10.3390/nu8070433. PMID: 27438851.

Acknowledgment *UAB and UNC-CH.*