KRAS G12C as a Target of Naringenin for Inducing Cell Death in NCI-H23 Cells

Abani Kumar Patar, Lakhon Kma, Jitul Barman, Shekhar Ghosh, Taranga Jyoti Baruah*

INTRODUCTION

Non-small cell lung cancer (NSCLC) is one of the most common forms of cancer worldwide and has a mortality rate of almost 26%.[1] The most commonly altered proteins in the adenocarcinomas subtype of NSCLC include epidermal growth factor receptor (EGFR), Kirsten rat sarcoma (KRAS), the Phosphatidylinositol 3-kinase/Protein Kinase B (PI3K/Akt) pathway components, Mesenchymal epithelial transition (MET), ROS proto-oncogene 1 (ROS1) and B-Raf protooncogene (BRAF). These proteins also happen to be the prime candidates for targeted therapy.[1] There are FDA approved inhibitors of EGF-R, the PI3K/Akt pathway components, and ROS1 that are being used as a replacement for chemotherapy.[2] Although tumours respond well to the initial treatment, the cancer cells invariably attain resistance against these inhibitors. Resistance is associated with mutations in MET, KRAS, and epithelial to mesenchymal transition (EMT).[2] Central to the KRAS-PI3K/Akt signalling chain is the activation of the Akt protein that enables tumour resistance by suppressing apoptosis, promoting the expression of anti-apoptotic proteins.[3] In H23 cells, the KRAS G12C mutant promotes the PI3K/Akt signalling. That makes H23 cells resistant to regular chemotherapy treatments.[6] Recently, sotorasib has become the first KRAS specific inhibitor to be approved by the FDA against NSCLC. On the flip side, almost 70% of the patients in the clinical trial showed adverse side effects that included grade 3 and grade 4 events and a median survival of 12 months.[7]

We turned to flavonoids to look for a safer alternative to sotorasib. We chose the flavonoid naringenin (NGN) as it has reported anti-cancer activities. NGN is a component of commonly consumed fruits and vegetables. Studies have proven NGN to be safe for oral consumption.[19] We observed from our in-silico study that NGN bound steadily to the KRAS G12C mutant. Our in-vitro studies showed the occurrence of apoptosis in the NGN treated H23 cells. In this regard, we propose NGN as a potential inhibitor of activation.[4] KRAS-PI3K/Akt signalling promotes epithelial to mesenchymal transition (EMT), which allows cancer cells to achieve metastatic potential, facilitating resistance to chemotherapy drugs.[3]

ABSTRACT

Background: Naringenin (NGN) is a commonly available flavonoid in the citrus fruits. We have previously shown that NGN is cytotoxic to the non-small cell lung cancer (NSCLC) cell line NCI-H23 (H23). Objectives: To check whether NGN could bind to the Kirsten rat sarcoma (KRAS) G12C mutant and cause its inhibition to promote apoptosis in H23 cells. Materials and Methods: NGN was docked with mutant KRAS protein followed by molecular dynamics simulation. HDOCK was used to analyse the influence of NGN on the KRAS and PI3K protein-protein docking. We checked the ultramorphological structure of the cells. A 2D-QSAR study was carried out to predict the activity of NGN. Results: We observed that NGN bound stably to the mutant KRAS. NGN showed steady RMSF and RMSD values, good structural stability, and favourable MM/PBSA values. NGN interfered in the binding of KRAS and PI3K. NGN treated cells showed hallmarks of apoptotic cell death. The predictive pIC50 value was found to be 7.39 for NGN against KRAS. NGN cleared all the drug filters. Conclusion: We conclude that NGN could bind to the mutant KRAS potentially inhibiting KRAS. That affects the PI3K/Akt pathway activation leading to apoptosis in the NCI-H23 cells.

Keywords: NSCLC, KRAS, Naringenin, Docking, Apoptosis, QSAR.
KRAS G12C and advocate further studies on its inhibitory effect on the G12C mutant of KRAS.

**MATERIALS AND METHODS**

**Preparation of Ligand and Protein for Docking**

The 3D structure of NGN was downloaded from the PubChem server.[19] The Open Babel software was used to convert the .sdf files into .pdb format.[20] The Autodock application of MGL tools was then utilized to generate the final .pdbqt file format. The .pdb file for the KRAS wild type and mutant proteins were converted to .pdbqt files using the Autodock function.[21]

**Docking using Autodock 4.0**

For carrying out the docking investigation, the amino acids involved in protein functioning were incorporated within the grid box set at 60 × 60 × 60 Å (x, y, and z). The grid spacing was kept at 0.5 Å. Autodock analysis was carried out.[21] Discovery studio software was used to obtain 2D images of the flavonoid-protein docking.

**Conserved Domain**

We utilised the Conserved domain database of the NCBI website[12-15] to analyse the amino acids involved in carrying out critical functions of the KRAS protein as per the protocol mentioned by Yang et al.[16]

**Molecular Dynamics Simulation**

The molecular dynamics (MD) simulation has been conducted for the NGN-KRAS complex using GROMACS 2018.3 for a time scale of 100 ns. The AMBER99SB-ILDN force field[17] was used to perform the simulation. Force field topology of ligand and protein was obtained using ACPYPE and pdb2gmx programs respectively.[18] A 1 nm water cube was used to solvate the protein-ligand complex. The system was neutralised using 0.15 M NaCl. The energy minimization was performed using the steepest descent and conjugate gradient for 50,000 steps. Finally, 100ns of production MD run was performed. The pressure of the protein-ligand system was maintained at 1 bar. The analyses were performed using GROMACS 2018.3 package[19] and, the plots were viewed using GRACE.[19]

**MM/PBSA based free Energy Calculation**

The MM/PBSA calculation for determining binding energy was performed using the g.mmpbsa tool was used. A python script MmPbsaStat.py, which is provided in the g_mmpbsa package, was used to calculate the binding energy components of protein-ligand binding.[20]

**Protein-protein Docking Study**

We performed protein-protein interaction studies of the mutant KRAS and the Ras Binding Domain (RBD) of PI3K protein in the absence and presence of NGN, based on a previous study by Basu et al.[21] with minor modifications. We used the HDOCKER webserver for carrying out the study.[22]

**Cell Culture**

We procured the NSCLC cell line NCI-H23 (H23) from the National Centre of Cell Science, Pune, India. The cells were maintained in a culture media containing RPMI 1640 (Invitrogen, USA), 2mM L-glutamine (Invitrogen, USA), 10% fetal bovine serum (FBS) (Himedia, India) and 0.1% antibiotics (Invitrogen, USA).

**Ultrastructural Studies**

To check for apoptosis, we performed the ultrastructure analysis of the H23 cells. We followed the protocol of Hayat et al.[27] with minor modifications for preparing the cells for ultrastructural study. At the end of the 24 hr treatment period, we trypsinized the cells and washed them with PBS. Cell fixation was achieved with Karnovsky’s fixative and 1% osmium tetroxide followed by dehydration. Cells were embedded in a mixture of embedding medium and propylene oxide followed by sectioning and staining with uranyl acetate and viewed under Transmission Electron Microscope (TEM).

**2D-Quantitative-structure activity relationship (QSAR) study**

For this QSAR study, a dataset of 37 compounds with inhibitory activity (IC_{50}) against the KRAS protein and also a structural file of NGN was retrieved from the ChEMBL database. The IC_{50} value of inhibitors was converted to the pIC_{50}(-LogIC_{50}). The conversion of 2D structure to 3D by Marvin Sketch software. Determination of 2D descriptors byPaDEL- Descriptor software version 2.2.0 was performed.[28] The data pretreatment, dataset division, model building, model validation, and prediction steps of QSAR studies were performed using the Drug Theoretics and Cheminformatics (DTC)-QSAR software. Dataset was divided into training and test datasets by applying Kennard-Stone’s algorithm.[29] The QSAR model, generated by the multiple linear regression (MLR) analysis, was validated and used for the prediction of the pIC_{50} value of inhibitors as well as NGN. Internal and external validation was carried as mentioned by Tropsha.[30] Moreover, a Y-Randomization test was performed to ensure the robustness of the developed model.

**Drug likeliness analysis**

NGN was checked for its drug-likeness ability using the Swiss ADME webserver.[31]

**RESULTS**

From our docking study, we observed that NGN had a better binding affinity to the KRAS G12C mutant protein as compared to the wild type KRAS protein (Table 1, Figure 1). NGN formed several hydrogen bonds with both the wild type and mutant KRAS proteins. The molecular dynamic simulation showed a gradual reduction of the system’s potential energy during the time period of simulation (Figure 2a). The solvent accessible surface area (SASA) of the complex remained approximately at 90 nm² during the time period of the simulation (Figure 2b). The radius of gyration (Rg) also stayed constant at 1.55 nm during the entire period of the simulation (Figure 2c). The Root mean square deviation (RMSD) values of the Cα carbons of the 4LUC (G12C KRAS) protein showed a steady RMSD value of 0.1 nm beyond 40 ns (Figure 3a). The RMSD values of the complex when NGN was the reference alignment (RMSD) values of the Cα carbons of the NGN-KRAS complex using GROMACS 2018.3 for a time scale of 100 ns. The AMBER99SB-ILDN force field[17] was used to perform the simulation. Force field topology of ligand and protein was obtained using ACPYPE and pdb2gmx programs respectively.[18] A 1 nm water cube was used to solvate the protein-ligand complex. The system was neutralised using 0.15 M NaCl. The energy minimization was performed using the steepest descent and conjugate gradient for 50,000 steps. Finally, 100ns of production MD run was performed. The pressure of the protein-ligand system was maintained at 1 bar. The analyses were performed using GROMACS 2018.3 package[19] and, the plots were viewed using GRACE.[19]

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**Table 1: Docking details of NGN with the KRAS wild type and the KRAS G12C mutant protein.**

| Sl. No | Protein       | PDB ID | Binding energy (kcal/mol) | Inhibition constant (μM) |
|-------|---------------|--------|--------------------------|-------------------------|
| 1     | KRAS protein  | 4LPK   | -7.41                    | 3.72                    |
| 2     | Mutant KRAS protein | 4LUC | -7.74                    | 2.11                    |
From our protein-protein docking studies (Table 3), we observed that the presence of NGN caused a lowering of the docking score between the mutant KRAS and the RBD of the PI3K 110α subunit. The ultramorphological analysis showed the presence of the standard apoptotic markers in the NGN treated cells like chromatin condensation, membrane blebbing, apoptotic body formation, cytoplasmic vesicle formation and nuclear fragmentation (Figure 5).[32] The untreated H23 cells didn’t show any of the apoptotic markers.

Based on the training dataset containing 26 inhibitors (Table 4), QSAR model was developed using 2D descriptors calculated solely from the structure of these chemical compounds. The QSAR model in terms of MLR was generated as: pIC$_{50}$ = -3.4957 - 3.3411 * AATSC7s - 0.0009 * ATSC6m + 0.1911 * AATSC0v. The QSAR model indicated that the dependent variable, pIC$_{50}$, has a significant correlation with the three descriptors namely AATSC7s, ATSC6m, and AATSC0v. As observed in the response plot (Figure 6a), a good correlation is evident between the experimentally observed pIC$_{50}$ values and the predicted pIC$_{50}$ values ranging from 5.07 to 7.94 and from 6.76 to 7.60 in the case of training and test dataset respectively. The statistical metrices $Q^2$, $R^2$, $R^2_{\text{adj}}$ are...
and SEE relating to the internal predictability quality of the model showed the score of 0.8221, 0.8761, 0.8592 and 0.3427 respectively. In Y-Randomization test against the model, average R² value of 0.152349 and average Q² value of 0.5126, average Rm² 2F2 = 0.6713, F1 = 0.6713, external validation of the model, results obtained were shown the score of 0.8221, 0.8761, 0.8592 and 0.3427 respectively. In

DISCUSSION

We have previously shown that NGN had a cytotoxic effect upon the H23 cells with an IC₅₀ value of approximately 100 μM. We have demonstrated the inhibition of colony formation of H23 cells by NGN and the lack of any adverse effects on the fibroblast cell line HEK293T that harbours a wildtype KRAS.[33-34] NGN is bound to several critical amino acids in the KRAS protein. From the conserved sequence database of NCBI, the amino acid residues ALA 18, ASN 116, LYS 117, ASP 119, LEU 120, ALA 146, and LYS 147 were found to be present in the GTP/Mg²⁺ binding site. Obstruction of the binding of GTP to the KRAS protein has proven to inhibit the actions of the downstream effector proteins like RAF and PI3K.[35] The gradual reduction of the system's energy indicated that the system was close to its natural structure (Figure 2a).[36] The steady values of the constant RMSD value in the range of 0.1 nm indicates a good binding interaction between NGN and the mutant KRAS. The higher RMSF values of the amino acids in position range of 60-73 indicated that the constant RMSD value in the range of 0.1 nm indicates a good binding interaction between NGN and the mutant KRAS. The higher RMSF values of the amino acids in position range of 60-73 indicated that the system was close to its natural structure (Figure 2a).[36] The steady values of

![Table 2: Energies associated with mutant KRAS and NGN binding.](image)

![Table 3: Protein-protein binding energy obtained in the absence and presence of NGN.](image)

![Figure 5: TEM micrographs showing (a) untreated cell showing intact plasma membrane (PM), evenly distributed chromatin (EC), intact nuclear membrane (NM), (b) NGN treated cell showing nuclear fragmentation (NF), chromatin condensation (CC), vesicle formation (V), membrane blebbing (MB) and apoptotic bodies (AP).](image)

![Table 4: Observed pIC₅₀, 2D descriptor, and predicted pIC₅₀ values of training and test dataset inhibitors of KRAS.](image)
higher flexibility of those residues. These amino acids with higher flexibility comprise the GEF interaction site. Considering that higher RMSF values have correlated with lower catalytic efficiency, our RMSF results may be indicative of impaired nucleotide exchange capacity of the mutant KRAS protein when bound to NGN. We observed the regular presence of 5 H-bonds between NGN and the mutant KRAS protein. Previous workers have reported almost the same or lower number of H-bond interactions between the mutant KRAS and the compounds under study. The MM/PBSA results confirmed the favourable binding, thermodynamic stability and inhibitory potency in the binding between KRAS and NGN.

The docking results between the mutant KRAS and the Receptor binding domain (RBD) of PI3K indicated the hampering of the binding interactions between the mutant KRAS with the RBD of the PI3K 110α subunit. Our previous study confirms this inference as we had observed a significant reduction in the levels of pAkt protein in the NGN treated group compared to the untreated cancer cells. The presence of apoptosis markers in the NGN treated cells correlates with our previously reported increase in the caspase-3 activity in the NGN treated H23 cells. Thus we can hypothesize that treatment with NGN could potentially bind with KRAS, inhibiting the activation of the PI3K/Akt pathway and promoting apoptotic cell death in H23 cells.

In this study, AATSC7s, ATSC6m, and AATSC0v, all 2D autocorrelation descriptors, were found to be highly significant. The model highlights the positive contribution of AATSC0v and the negative contribution of AATSC7s and ATSC6m on the activity. The pIC50 value is augmented in response to the increase of AATSC0v whereas it is diminished in response to the increase of AATSC7s and ATSC6m. The acceptability of the model in terms of stability, predictive ability, and fitness can be ascertained from the high values of $R^2$ and $Q^2_{(LOO)}$ and low value of SEE. It is also clear that the external predictive quality of the model is good as confirmed from the results of statistical analysis of the test dataset. The Y-Randomization test involves the repeating of a process in which the dependent variable vectors are shuffled randomly while keeping the independent variable vectors unaltered to build new models.

The lower value of average $R^2$ and average $Q^2_{(LOO)}$ of these new models indicates that the current model is robust and not an outcome of chance. Interestingly, the predictive pIC50 value of NGN computed by the QSAR model is relatively higher as well as comparable to observed pIC50 value of selected KRAS inhibitors in this study, implying the significant possible action of NGN against the KRAS activity. The ability of NGN to clear all the drug filters further highlighted the suitability and safety profile of NGN for future clinical success.

CONCLUSION

Our study indicates that the cytotoxicity of NGN towards H23 cells can be attributed to NGN potentially binding to the mutant KRAS G12C protein harboured in the H23 cells. NGN caused interference in the binding between the KRAS G12C protein and the PI3K protein inhibiting the latter’s activity. That lead to PI3K/Akt pathway inhibition and the subsequent apoptotic activity in the NGN treated cells. NGN showed a favourable pIC50 value against KRAS protein, and NGN also cleared all the drug likeliness filters. Thus NGN should be further studied as a potential candidate for the treatment of G12C KRAS harbouring cancers.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.
ABBREVIATIONS
NSCLC: Non Small Cell Lung Cancer; EGFR: Epidermal Growth Factor Receptor; KRAS: Kirsten Rat Sarcoma; PI3K/ Akt: Phosphatidyl Inositol 3 kinase/Protein Kinase B; MET: Mesenchymal Epithelial Transition; ROS1: ROS proto-oncogene1; BRAF: B-Raf Proto-oncogene; EMT: Epithelial to Mesenchymal Transition; NGN: Naringenin; RMSD: Root Mean Square Deviation; RMSF: Root Mean Square Fluctuation; SASA: Solvent Accessible Surface Area; QSAR: Quantitative-Structure Activity Relationship; MM/PBSA: Molecular Mechanics energies combined with the Poisson–Boltzmann or generalized Born and Surface Area continuum solvent.

REFERENCES
1. Majed U, Manochakian R, Zhao Y, Lou Y. Targeted therapy in advanced non-small-cell lung cancer. Signal Transduct Target Ther. 2019 Dec 17;4(1):1-14.
2. Molina-Arós M, Moore C, Rana S, Maldegem van F, Mugura E, Romero-García P, Herbert D, Horswell S, Li LS, James MR, Hancock DC. Downward J. Development of combination therapies to maximize the impact of KRAS-G12C inhibitors in lung cancer. Sci Transl Med. 2019 Sep 18;11(150):1-33. doi: 10.1126/scitranslmed.aaw7799, PMID 31534020.
3. Cuesta C, Árvalo-Alamedra C, Castellano E. The importance of being PI3K in the RAS signaling network. Genes (Basel). 2021 Jul 19;12(7):1-40. doi: 10.3390/genes12070994, PMID 34356110.
4. Sunaga N, Shames DS, Girard L, Peyton M, Larsen JE, Imai H, Suh J, Sato M, Yanagitani K, Kaira Y, Xie Y, Gazdar AF, Mori M, Minna JD. Knockdown of oncogenic KRAS in non-small cell lung cancers suppresses tumor growth and sensitizes tumor cells to targeted therapy. Mol Cancer Ther. 2011 Feb;10(2):336-48. doi: 10.1158/1535-7163.MCT-10-0781, PMID 21306991.
5. Hong DS, Fakhig MG, Strickler HD, Desai J, Durum GA, Shapiro GI, Falchook GS, Price TJ, Sacher A, Denlinger BS, Bang YJ, Gq KJ, Krauss JC, Kubko YJ, Cuveller AL, Park K, Kim TW, Barlesi F, Munster PN, Burns TF, Coveler AL, Park K, Kim TW, Barlesi F, Munster PN, Burns TF, Merci-Berstam F, Henary H, Nang J, Namgmentamann G, Chin J, Houk BE, Cano J, Lipford JP, Friberg G, Liu F, Govindan R, Li BT. KRASG12C inhibition with Sotoretasib in advanced solid tumors. N Engl J Med. 2020 Sep 24;383(13):1207-17. doi: 10.1056/NEJMoa1917239, PMID 32955176.
6. Rebello CJ, Beyl RA, Lertora JJL, Greenway FL, Ravussin E, Ribnicky DM, Lee S, Polonsky KS, Tamborlane WV, Coven RE. Weight loss with low-calorie research diet improves glucose tolerance in overweight/obese adults with metabolic syndrome: results of a randomized, controlled, single-ascending-dose clinical trial. Diabetes Obes Metab. 2020 Jan;22(1):91-98. doi: 10.1111/dob.13886, PMID 31488836.
7. Kim S, Thiesse PA, Bolton EE, Chen J, Thiessen PA, Bolton EE, Chen J, Fu G, Gindulyte A, Han L, He J, He S, Thanki N, Wang Z, Yamashita RA, Zhang D, Zheng C, Bryant SH, Geer L, Gwadz M, Hurwitz DI, Lanczycki CJ, Lu F, Marchler GH, Song JS, Thanki N, Wang Z, Yamashita RA, Zhang D, Zheng C, Geer LY, Bryant SH. CDD/SPARCLE: the conserved domain database and tools for protein domain analysis. Curr Protoc Bioinformatics. 2012;73(1):43:14 dela. doi: 10.1002/9780470094646.vw327a31, PMID 19672399.
8. Abraham MJ, Murtola T, Schulz R, Pall S, Smith JC, Hess B, Lindahl E, Lindahl E. GROMACS: high performance molecular simulations combined into a new versatile system. J Chem Theory Comput. 2015 Sep 1;11(9):3102-21. doi: 10.1021/acs.jctc.5b00450, PMID 26276424.
9. Kikov AA, Castellano E. PI3K: A crucial piece in the RAS signaling puzzle. Cold Spring Harb Perspect Med. 2016 Jan 1;6(1):1-19. doi: 10.1101/cshperspect.a031450, PMID 28847905.
10. O’Boyle NM, Banck M, Sweet AJ, Vranken WF. ACPYPE, AnteChamber P. Ython parser for Biopython. Presented at the 2004 PLoS Comput Biol 1;1(6):e67. doi: 10.1371/journal.pcbi.0010067, PMID 15426582.
11. Abraham MJ, Murtola T, Schulz R, Pall S, Smith JC, Hess B, Lindahl E, Lindahl E. GROMACS: high performance molecular simulations combined into a new versatile system. J Chem Theory Comput. 2015 Sep 1;11(9):3102-21. doi: 10.1021/acs.jctc.5b00450, PMID 26276424.
12. Abraham MJ, Murtola T, Schulz R, Pall S, Smith JC, Hess B, Lindahl E, Lindahl E. GROMACS: high performance molecular simulations combined into a new versatile system. J Chem Theory Comput. 2015 Sep 1;11(9):3102-21. doi: 10.1021/acs.jctc.5b00450, PMID 26276424.
13. Abraham MJ, Murtola T, Schulz R, Pall S, Smith JC, Hess B, Lindahl E, Lindahl E. GROMACS: high performance molecular simulations combined into a new versatile system. J Chem Theory Comput. 2015 Sep 1;11(9):3102-21. doi: 10.1021/acs.jctc.5b00450, PMID 26276424.
14. Abraham MJ, Murtola T, Schulz R, Pall S, Smith JC, Hess B, Lindahl E, Lindahl E. GROMACS: high performance molecular simulations combined into a new versatile system. J Chem Theory Comput. 2015 Sep 1;11(9):3102-21. doi: 10.1021/acs.jctc.5b00450, PMID 26276424.
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This work checked the possibility of naringenin docking with KRAS G12C protein to promote apoptosis in H23 cells. Our in-silico analysis showed the steady binding of naringenin with the mutant KRAS G12C protein. This interaction lead to the promotion of apoptosis in the H23 cells. 2D-QSAR analysis also showed a favourable pIC_{50} value for naringenin against KRAS protein.