Genes & Diseases (2023) 10, 2229–2232

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RAPID COMMUNICATION

Exploring the anti-cancer effects of *Panax notoginseng* through network pharmacology and molecular modeling

The second leading cause of death worldwide is cancer. Cancer is a general term that refers to a highly diverse and complex set of over 200 diseases where abnormal cells grow uncontrollably and avoid death.[1] These diseases can start almost anywhere within the body and may develop the ability to invade nearby tissues and spread. Every cancer is the byproduct of a unique combination of various genetic and environmental factors.[1] Even within the same individual, different cells may possess distinct cancerous mutations which can mutate further over time. Despite this inherent heterogeneity, the most common treatments for cancer are surgery, chemotherapy, and radiation.[1]

Panax notoginseng (PNG) [(Burkill) F. H. Chen ex C. H. Chow] has been used in traditional Chinese medicine for over 400 years to treat disorders related to blood circulation safely. More recently, *PNG* has shown promise in cancer prevention, treatment, and recovery. Several studies have shown the usefulness of specific *PNG* compounds in treating different types of cancer, and some have suggested the underlying mechanisms for these effects, but the detailed inner workings are still being uncovered.[2]

In this study, a systematic network pharmacology (NP) approach was employed to identify the important anti-cancer components of *PNG* and explore their potential mechanisms in numerous cancer subtypes. The compounds of *PNG* were collected and screened, and their protein targets were predicted (Table S1, S2). Known protein targets of cancer were collected and intersected with the targets of the *PNG* compounds to identify the common target proteins (Fig. 1AI; Fig. S1 and Table S3). A protein–protein interaction network was constructed from the common targets, and then the pathways related to these targets were collected and enriched (Fig. 1AII; Fig. S2 and Table S4). A target-(pathway)-target network was created to connect the common protein targets through their shared pathways. This network was then grouped into modules by a topological analysis, where the targets with closely related pathways were clustered to form subnetwork structures (Fig. 1AII; Fig. S3 and Table S5). The pathways were searched to obtain relevant disease categories and a contribution scoring algorithm was used to assess the fraction that each module contributed to different cancer subtypes (Fig. 1AII; Eq. S1 and Table S6, S7). A target importance score calculation was utilized to rank and identify the significant targets within each module (Fig. 1AIII; Equ. S2 and Table S8, S9). Nineteen cancer subtypes were placed into groups based on their module contribution scores and the top protein targets of each module were listed (Fig. 1AIII and Table S10). The relationships between the compounds, targets, pathways, and diseases were then established (Table S11), and the top targets with their ligand compounds were used to build a compound-target network (Fig. 1AIII; Fig. S4). The number of cancer subtypes that the top proteins and associated compounds were involved in was determined (Table S12). Molecular docking simulation was used to validate the binding of these core compounds to their key targets (Fig. 1B; Fig. S5, 6 and Table S13). Nearly all the best-scoring compounds in the molecular docking analysis belonged to a class of compounds called saponins. Ginsenosides are saponins found exclusively in ginseng species belonging to the genus Panax (Fig. S7).[2]

According to our molecular docking results, the saponin daucosterol bound to the BH3 domain of BCL2L1 with an affinity of −8.7 kcal/mol (Fig. 1BI). We also found that (20S)-ginsenoside Rh2 bound within the BH4 domain of BCL2L1 with an affinity of −8.7 kcal/mol (Fig. 1BII). BCL2L1 is an anti-apoptotic member of the BCL-2 family that is frequently overexpressed in different cancers and can contribute to therapeutic resistance.[3] FGF1 activates several signaling cascades that are important in cell proliferation, migration, and angiogenesis.[4] Heparin increases the stability of FGF1

Peer review under responsibility of Chongqing Medical University.

https://doi.org/10.1016/j.gendis.2023.02.022

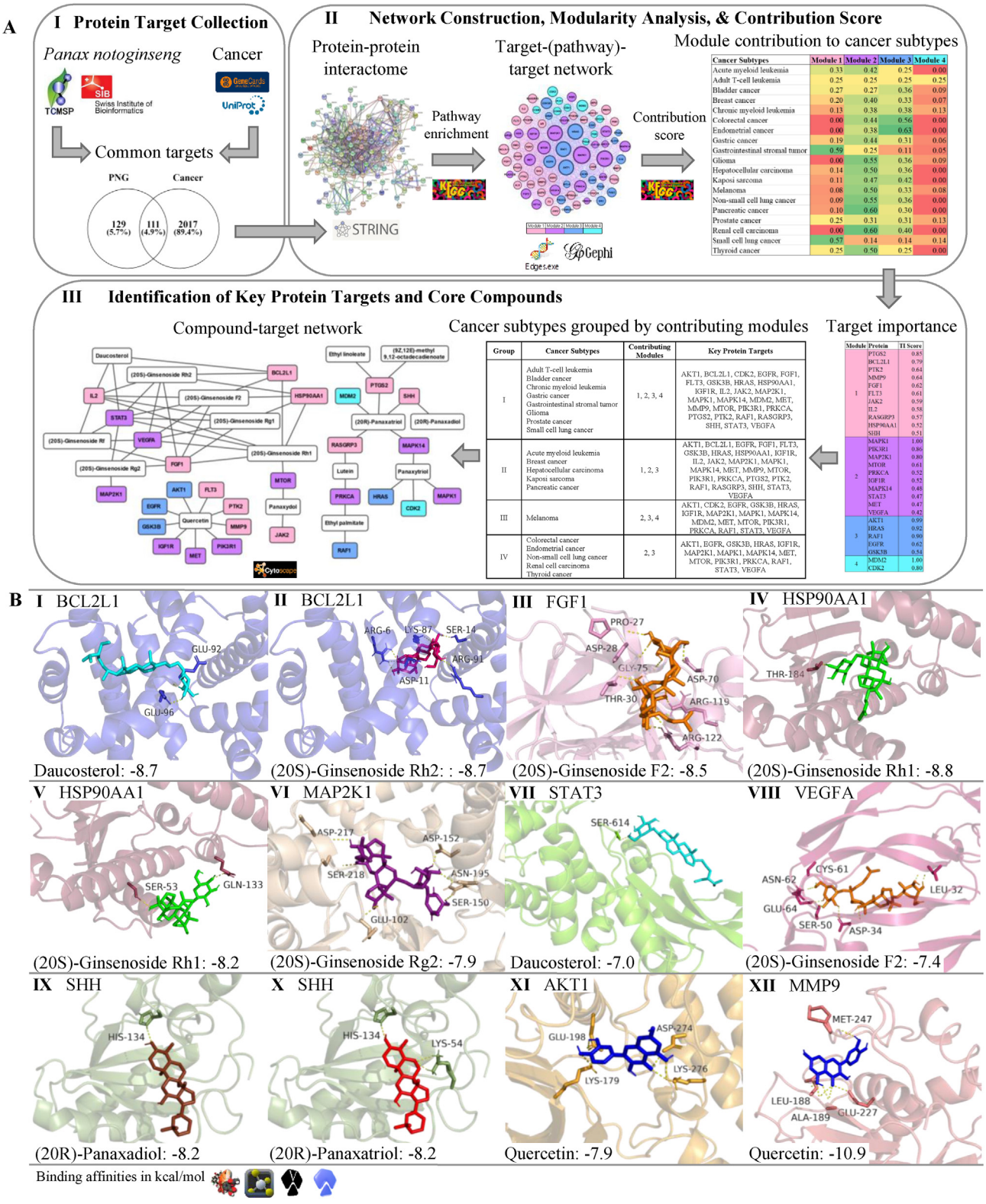

Figure 1 Network pharmacology analysis of *Panax notoginseng (PNG)* to identify key protein targets and core compounds. (**A**) (**I**) The chemical components of *PNG* were retrieved and filtered. The protein targets of the screened compounds were predicted. The protein targets related to cancer were gathered and cross-referenced. The common targets of *PNG* and cancer were identified. (**II**) These common targets were used to build a protein—protein interaction network. Pathways related to these targets were obtained and enriched. The combinations of target proteins within related pathways were used to build a target-(pathway)-target network, where the nodes represent target proteins and the edges connecting them represent the common pathways. This network was separated into subnetworks called modules based on target—pathway interactions. A contribution scoring algorithm was used to

and protects the growth factor from degradation.[4] (20S)-Ginsenoside F2 bound to the heparin-binding pocket of FGF1 with an affinity of −8.5 kcal/mol (Fig. 1BIII). (20S)-Ginsenoside Rh1 bound to the ATP-binding site on the stress-inducible molecular chaperone HSP90AA1 with an affinity of −8.8 kcal/mol (Fig. 1BIV). Disrupting ATP binding and hydrolysis leads to ubiquitination and proteosome-mediated degradation of oncogenic client proteins.[5] Additionally, we found that (20S)-ginsenoside Rh1 bound to the cdc37 binding site on HSP90AA1 with an affinity of −8.2 kcal/mol (Fig. 1BV). Cdc37 is important for the folding and maturation of cyclin-dependent kinases.[5] MAP2K1 is a dual specificity tyrosine and threonine kinase involved in the RAS/RAF/MEK/ERK signaling cascade. Once activated, ERK promotes cell survival through transcriptional up-regulation of genes involved in proliferation, invasion, and metastasis.[3] Our results indicate that (20S)-ginsenoside Rg2 can bind to MAP2K1 with an affinity of −7.9 kcal/mol in an allosteric site adjacent to the ATP-binding site known to be occupied by other type-III MAP2K1 inhibitors (Fig. 1BVI). Constant stimulation of the signal transduction and transcriptional activator STAT3 frequently occurs in different cancers and is associated with metastasis, chemotherapeutic resistance, and tumor survival.[3] Our study found that daucosterol bound to the SH2 domain of STAT3 with an affinity of −7.0 kcal/mol (Fig. 1BVII). Blocking the SH2 domain of STAT3 prevents the phosphorylation, dimerization, and nuclear translocation of STAT3, ultimately halting the transcription of target oncogenes.[3] VEGFA is a vascular endothelial growth factor that mediates angiogenesis by binding to VEGF receptors. Expression of VEGFA is up-regulated in a variety of cancer types in response to growth factors and hypoxia.[2,3] (20S)-Ginsenoside F2 bound to VEGFA with an affinity of −7.4 kcal/mol in a pocket with residues that have been previously associated with VEGFR2 binding (Fig. 1BVIII).

SHH is the most widely expressed and potent ligand involved in the initiation of the hedgehog signaling pathway.[3] Binding of active SHH to its receptor PTCH1 initiates the SMO signaling pathway by releasing repression of SMO by PTCH1. Activation of SMO stimulates the translocation of GLI transcription factor proteins into the nucleus which promotes gene expression.[3] Our docking results show that (20R)-panaxadiol and (20R)-panaxatriol each bind to SHH with an affinity of −8.2 kcal/mol in a location capable of disrupting SHH and PTCH1 interaction (Fig. 1BIX, BX). The key targets of the ubiquitous dietary flavonoid quercetin were all found to be involved in various aspects of the PI3K/AKT pathway. This signaling pathway is crucial for cell growth and survival regulation.[3] Therefore, unsurprisingly, disruption of this elaborate pathway has been observed in nearly all human cancers. Quercetin competing for the ATP-binding site of numerous kinases was paralleled in our results. Quercetin bound to AKT1 with an affinity of −7.9 kcal/mol (Fig. 1BXI).[3] Additionally, quercetin displayed a binding affinity of −10.9 kcal/mol for the S1′ subsite of MMP9 (Fig. 1BXII).[2] The PI3K/AKT pathway serves as a convergence point for many growth factor signals and plays an integral role in cancer growth and progression through its action on numerous substrates. Thus, targeting multiple proteins within this pathway with quercetin is an attractive cancer treatment strategy.

In summary, the core anti-cancer compounds of *PNG* were identified through comprehensive network analysis, and their key targets were investigated through molecular docking. The findings of this research confirmed and expounded the results of several previous studies and revealed novel insights into the therapeutic roles of different *PNG* compounds in treating various cancer subtypes. The results of our study warrant further *in vitro*, *in vivo*, and clinical studies to validate the proposed anti-cancer mechanisms of the *PNG* compounds.

Author contributions

E.M.T.: data collection, data analysis, manuscript writing, and figure preparation, M.K. and Y.X.: conceptualization; Y.X.: methodology, supervision, and manuscript editing. All authors reviewed and approved the manuscript.

Conflict of interests

The authors declare no competing interests.

Funding

This project was supported by funds from a Graduate Student Research Award from Cleveland State University (E.M.T.).

Data availability

Datasets used or analyzed during this study are available upon reasonable request from the corresponding author.

calculate the fraction that each module contributed to different cancer subtypes. (**III**) A centrality analysis was utilized to identify the important targets within each module and then the cancer subtypes were grouped based on their contributing modules. The core compounds and key protein targets were used to create a compound-target network. (**B**) Molecular docking images of key protein targets with core compounds. Hydrogen bonds are represented with yellow dotted lines and amino acid residues participating in these bonds are labeled accordingly. (**I**) BCL2L1 and daucosterol: −8.7 kcal/mol. (**II**) BCL2L1 and (20S)-ginsenoside Rh2: −8.7 kcal/mol. (**III**) FGF1 and (20S)-ginsenoside F2: −8.5 kcal/mol. (**IV**) HSP90AA1 and (20S)-ginsenoside Rh1: −8.8 kcal/mol. (**V**) HSP90AA1 and (20S)-ginsenoside Rh1: −8.2 kcal/mol. (**VI**) MAP2K1 and (20S)-ginsenoside Rg2: −7.9 kcal/mol. (**VII**) STAT3 and daucosterol: −7.0 kcal/mol. (**VIII**) VEGFA and (20S)-ginsenoside F2: −7.4 kcal/mol. (**IX**) SHH and (20R)-panaxadiol: −8.2 kcal/mol. (**X**) SHH and (20R)-panaxatriol: −8.2 kcal/mol. (**XI**) AKT1 and quercetin: −7.9 kcal/mol. (**XII**) MMP9 and quercetin: −10.9 kcal/mol.

Acknowledgements

We would like to thank Ehab Rizek for developing Edges Software which greatly assisted our data analysis. We also express gratitude to Theresa Nawalaniec, the Cleveland State University Sciences and Engineering Librarian, for her assistance in making Edges Software publicly available (https://engagedscholarship.csuohio.edu/scichem_softw/1/).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2023.02.022.

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30 December 2022
Available online 27 March 2023