


LETTER TO THE EDITOR

Open Access



The epitome of tailor-made short positively charged peptides against HCC via integrated pharmacology

Ki-Kwang Oh¹, Jung-A Eom¹, Kyeong Jin Lee¹, Goo-Hyun Kwon¹, Sang-Jun Yoon¹, Seol Hee Song¹, Jeong Ha Park¹, Jeong Su Kim¹, Dong Joon Kim¹ and Ki-Tae Suk^{1*} 

Dear Editor,

Recently, short positively charged peptides (SPCPs ≤ 500 Dalton) with positively charged residues (Arginine; R, Histidine; H, and Lysine; K) have been identified as alternative therapy with anti-cancer efficacy, which can penetrate plasma membrane to reach at target(s) [1]. The peptides are exposed to unfavorable pharmacokinetic properties such as hydrophilicity, high degradability, and short half-life in oral formulation. Alternatively, intra-tumoral injection (ITUI) is a promising strategy to improve the immunogenicity for anti-tumors [2].

Moreover, cancer cell membrane (CCM) is surrounded by phosphatidylserine (PS) with negatively charged ions in pH 6.2–6.9 [3]. The SPCPs can destroy the CCM with negatively charged residues, which is optimal to develop suitable agents. Thus, SPCPs obtained by R Package filtered to select significant SPCPs with stable physicochemical properties via the reliable web-based bioinformatics tools. The layout of this study was depicted in Fig. 1A.

(See figure on next page.)

Fig. 1 **A** The mining workflow of short positively charged peptides (SPCPs) of this study. **B** The number of 244 targets between SEA and STP. **C** The final 136 targets for the analysis. **D** The protein–protein interaction (PPI) networks. **E** The 16 signaling pathways related to hepatocellular carcinoma. **F** The number of 6 intersecting SPCPs in 5 key targets from SEA. **G** The number of 7 intersecting SPCPs in 5 key targets from STP. **H** The SPCPs–targets–signaling pathway (STS) networks. A: Alanine; F: Phenylalanine; H: Histidine; K: Lysine; S: Serine; T: Threonine; V: Valine. **I** The docking scores on 2 SPCPs (KFAH, and HFAK)–5 key targets (CTSB, CASP1, BIRC3, XIAP, and CASP8) via HPEPDOCK 2.0. Blue rectangles: the most significant conformers on SPCPs–key targets. **J** The CA-074—CTSB conformer via AutoDock. **K** The Belnacasan—CASP1 conformer via AutoDock. **L** The GDC-0152—BIRC3 conformer via AutoDock. **M** The GDC-0152—XIAP conformer via AutoDock. **N** The Z-IETD-FMK—CASP8 conformer via AutoDock. **O** The comparison of energy gap between two key SPCPs and standard compounds

*Correspondence:

Ki-Tae Suk

ktsuk@hallym.ac.kr

¹ Institute for Liver and Digestive Diseases, College of Medicine, Hallym University, Chuncheon 24252, Korea



© The Author(s) 2024. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

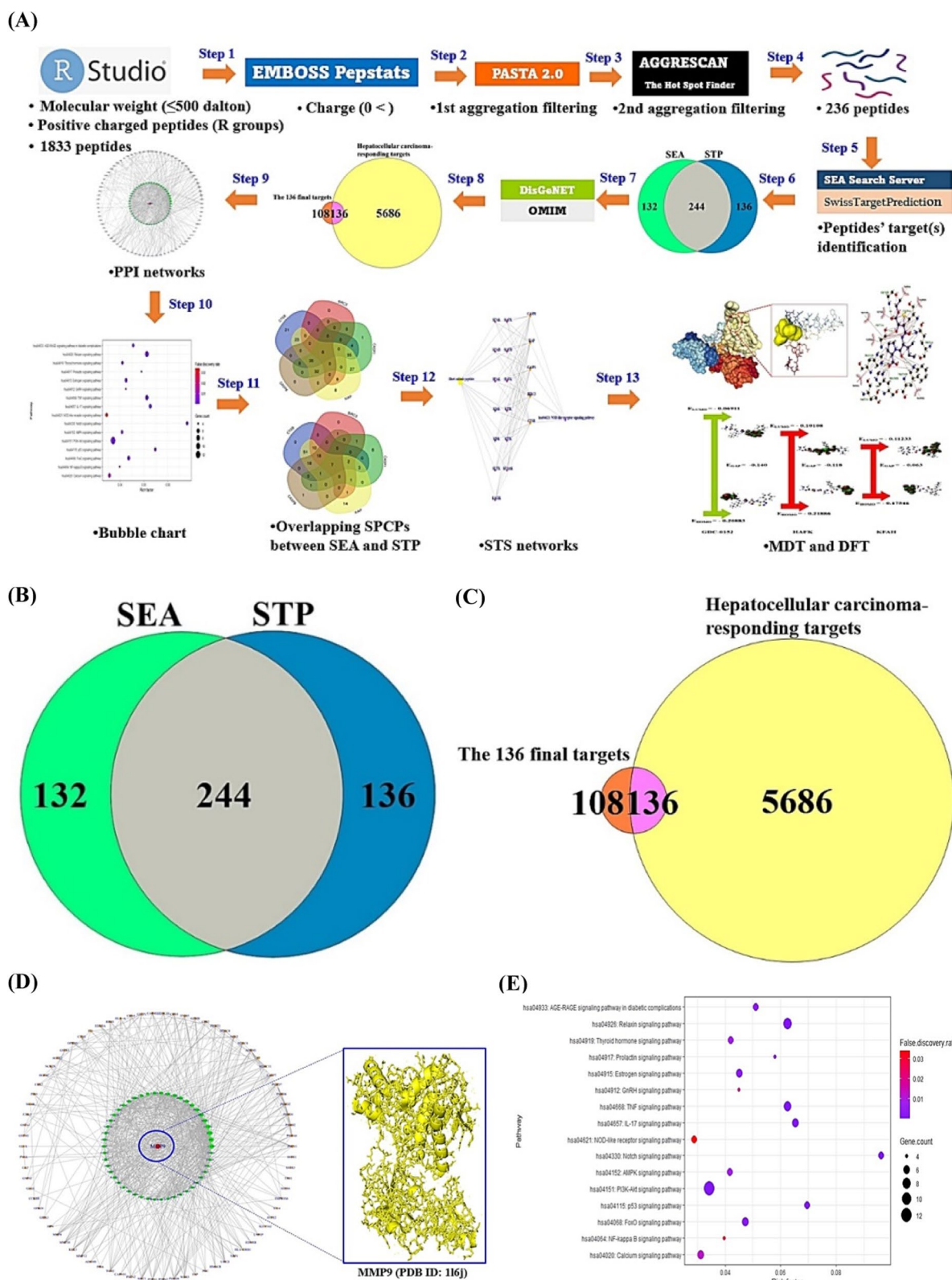


Fig. 1 (See legend on previous page.)

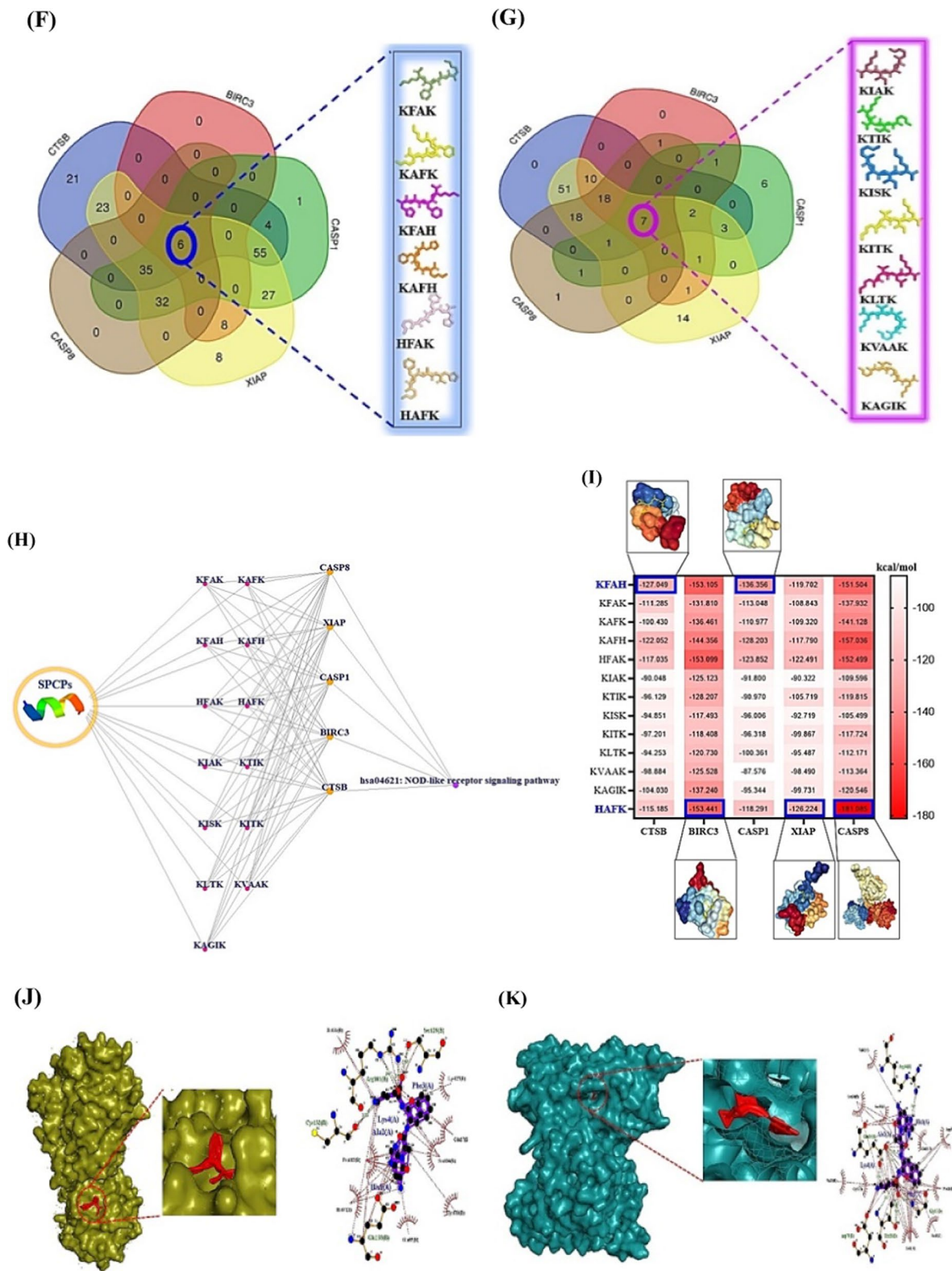


Fig. 1 continued

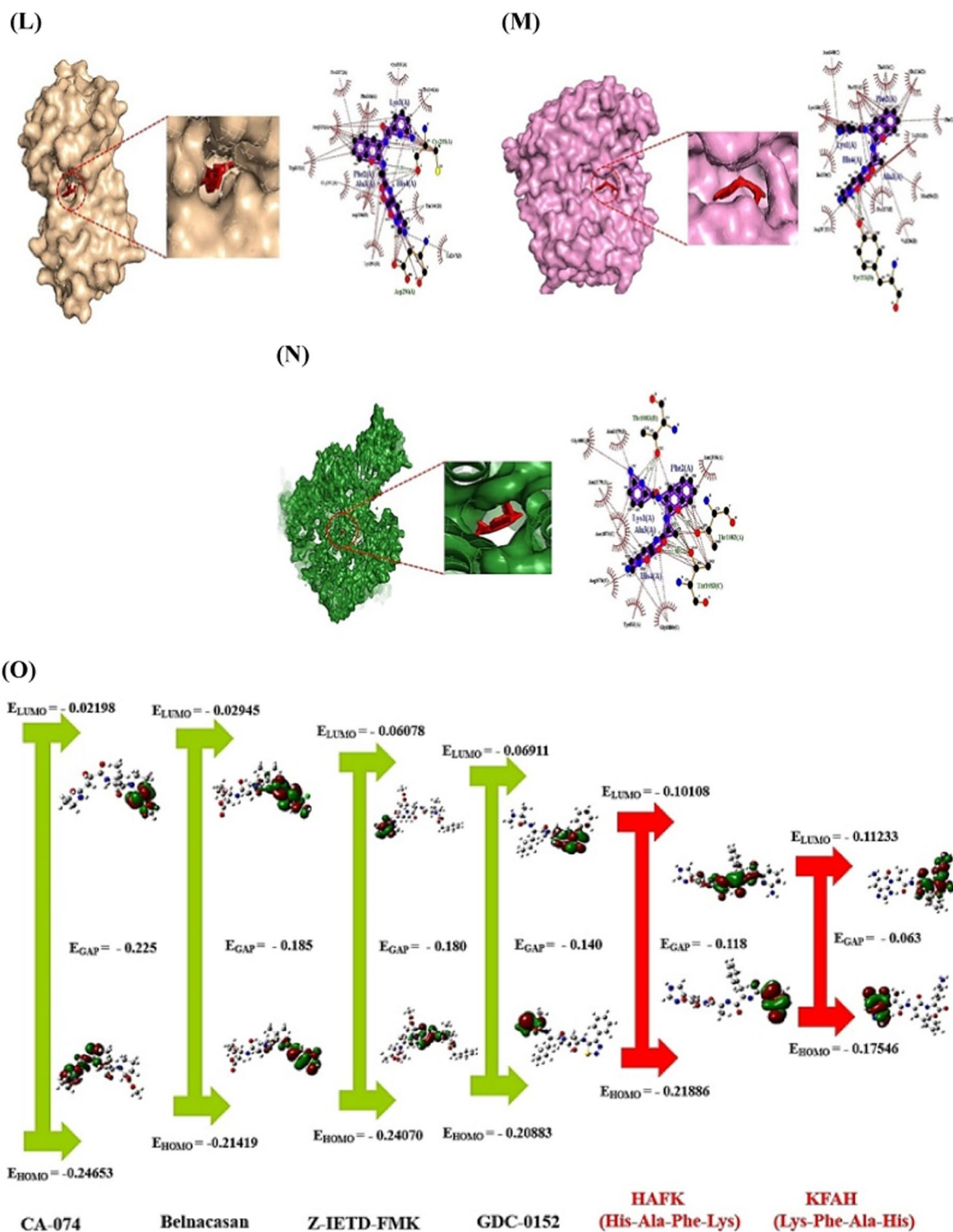


Fig. 1 continued

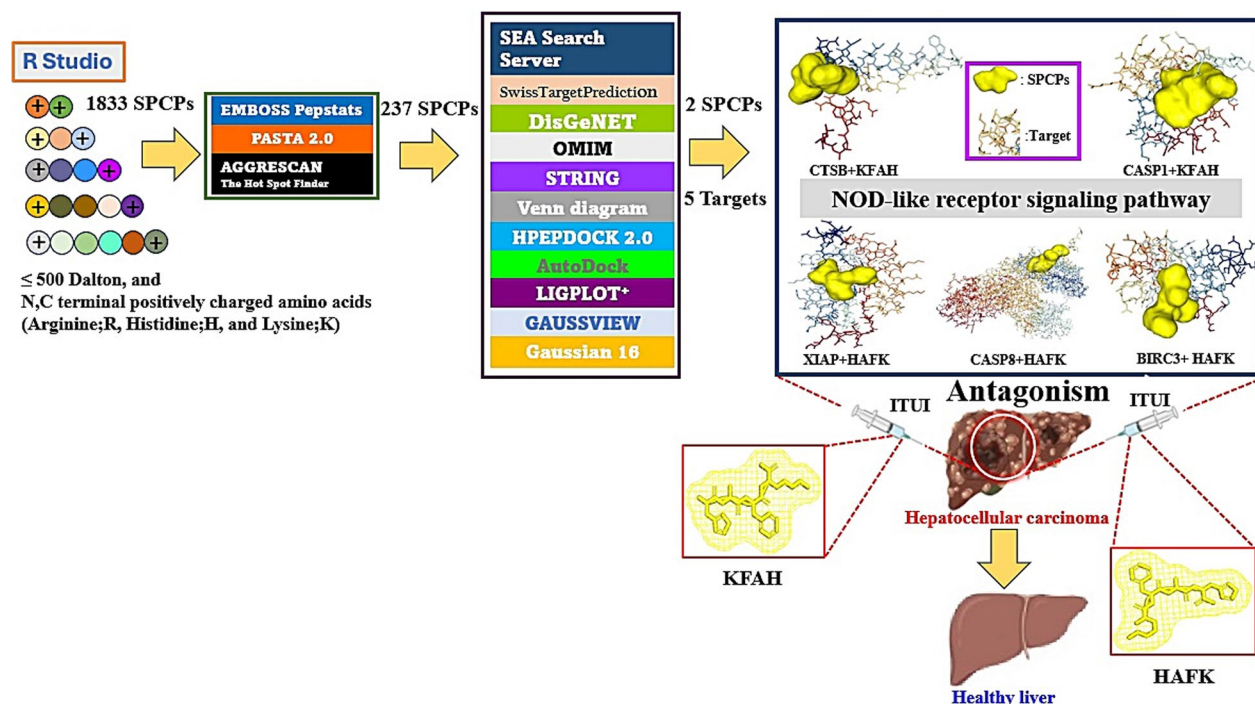


Fig. 2 The key summary of this study

Firstly, the definition SPCPs is as below.

- 1) Peptide(s) less than 500 Dalton (DA).
- 2) Peptides with cationic amino acids on N-terminal, and C-terminal.

The 1833 SPCPs (Additional file 1: Table S1) to be accepted by the two criteria were input to EMBOSS Pepstats (https://www.ebi.ac.uk/Tools/seqstats/emboss_pepstats/) to obtain the SPCPs with specific isoelectric point between 8 and 12. Then, SPCPs with non-aggregated propensity were filtered in PASTA 2.0 (<https://protein.bio.unipd.it/>), and AGGRESCAN ($-40 \leq \text{Na4VSS} \leq 60$) (<http://bioinf.uab.es/aggreSCAN/>). The 236 out of 1833 SPCPs was accepted by the filtering point (Additional file 1: Table S1).

Secondly, the targets on SPCPs were retrieved by Similarity Ensemble Approach (376 targets) and SwissTargetPrediction (380 targets), indicating that the overlapping targets (244 targets) might be significant elements in SPCPs (Additional file 2: Table S2) (Fig. 1B). The HCC-responding targets (5822 targets) were identified by DisGeNET and OMIM databases, compared with the 244 targets (Additional file 2: Table S2). The 136 targets were considered as therapeutic targets against HCC via SPCPs (Additional file 2: Table S2) (Fig. 1C).

Thirdly, the final 136 targets were put into STRING platform to represent the protein–protein interaction

(PPI) networks, thereby, 129 targets were interconnected directly to each one another (129 nodes, and 800 edges) (Fig. 1D). The highest-ranking target in the PPI networks was MMP9 with the greatest degree of value (46) (Additional file 3: Table S3). The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was represented 16 signaling pathways associated with the occurrence and progression of HCC (Fig. 1E) (Additional file 4: Table S4). It implied that NOD-like receptor signaling pathway with the lowest rich factor might be an antagonism to alleviate HCC. The 5 targets (BIRC3, CASP1, CASP8, CTSB, and XIAP) related to NOD-like receptor signaling pathway were considered as protein coding genes to combat HCC. More importantly, the 6 SPCPs has been intersected with the 5 targets, suggesting that the SPCPs might be significant effector(s) (Additional file 5: Table S5), (Fig. 1F). Likewise, the 7 SPCPs has been overlapped with the 5 targets, indicating that the SPCPs might be important agent(s) (Additional file 5: Table S5), (Fig. 1G). The SPCPs-targets-signaling pathway networks consist of 20 nodes and 83 edges, representing that the three components might function as therapeutic module (Fig. 1H).

Lastly, in the molecular docking test, the two SPCPs (KFAH, and HFAK) formed the most stable complex on the 5 key targets (Fig. 1I). The KFAH had the strongest affinity on CTSB, and CASP1, the HFAK bound most

stably to BIRC3, XIAP, and CASP8. The AutoDock program was employed to confirm its affinity with rigorously by comparison with standard drugs (Additional file 6: Table S6) (Fig. 1J–N). As a result, the two key SPCPs had more potent affinity than known ligands. Additionally, density functional theory (DFT) analysis was performed to clarify the aptness of the physico-chemical properties (Additional file 7: Table S7). The two SPCPs manifested that they have high chemical reactivity (Fig. 1O) and conformer stability.

In conclusion, the pinpointed SPCPs (KFAH, and HAFK) had more potency than existing drugs for the treatment of HCC, the mechanism of which was to dampen NOD-like receptor signaling pathway on the 5 targets. Thereby, the two key SPCPs are capable to exert to antagonism, supporting that they can be developed into ITUI formulation to evade the pharmacokinetic drawbacks in systemic circulation. The key summary of this study was unfolded in Fig. 2.

Abbreviations

R	Arginine
CCM	Cancer cell membrane
DA	Dalton
DFT	Density functional theory
χ	Electronegativity
E_{GAP}	Energy gap
FMO	Frontier molecular orbitals
η	Hardness
HCC	Hepatocellular carcinoma
HAFK	His-Ala-Phe-Lys
H	Histidine
ITUI	Intra-tumoral injection
KEGG	Kyoto Encyclopedia of Genes and Genomes
K	Lysine
KFAH	Lys-Phe-Ala-His
PS	PhosphatidylSerine
PPI	Protein–protein interaction
SPCP	Short positively charged peptide
SPCPs	Short positively charged peptides
S	Softness

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12967-024-05087-w>.

Additional file 1: Table S1. The profiling of short positively charged peptides (SPCPs).

Additional file 2: Table S2. The profiling of key targets in this study.

Additional file 3: Table S3. The degree of value on targets.

Additional file 4: Table S4. The description of 16 signaling pathways related to occurrence and progression of HCC.

Additional file 5: Table S5. The list of potential SPCPs on NOD-like receptor signaling pathway.

Additional file 6: Table S6. The docking score of key SPCPs and standard compounds.

Additional file 7: Table S7. The DFT parameters of two key SPCPs and three standard drugs.

Acknowledgements

Open access publishing facilitated by Hallym University, the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology, Korea Institute for Advancement of Technology, and Bio Industrial Technology Development Program funded by the Ministry of Trade, Industry and Energy (MOTIE, Korea).

Author contributions

Methodology, conceptualization, data curation, software, writing—original draft: KKO, Methodology, data curation, software: JAE, KJL, Formal analysis, validation: GHK, SJY, Methodology, data curation: SHS, JHP, Methodology, formal analysis: JSK, Supervision, investigation, project administration: DJK, Funding acquisition, conceptualization, writing—original draft, writing—review editing: KTS.

Funding

This research was supported by Hallym University Research Fund, the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (NRF2019R111A3A01060447 and NRF-2020R1A6A1A03043026), Korea Institute for Advancement of Technology (P0020622), and Bio Industrial Technology Development Program (20018494) funded by the Ministry of Trade, Industry and Energy (MOTIE, Korea).

Availability of data and materials

All data generated or analyzed during this study are included in this published article (and its Additional files).

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

There is no conflict of interest declared.

Received: 7 March 2024 Accepted: 11 March 2024

Published online: 15 March 2024

References

1. Bitler BG, Schroeder JA. Anti-cancer therapies that utilize cell penetrating peptides. Recent patents on anti-cancer drug discovery. *Recent Pat Anticancer Drug Discov.* 2010;5:99.
2. Nobuoka D, Yoshikawa T, Takahashi M, Iwama T, Horie K, Shimomura M, et al. Intratumoral peptide injection enhances tumor cell antigenicity recognized by cytotoxic T lymphocytes: a potential option for improvement in antigen-specific cancer immunotherapy. *Cancer Immunol Immunother.* 2013;62:639.
3. Alves AC, Ribeiro D, Nunes C, Reis S. Biophysics in cancer: the relevance of drug-membrane interaction studies. *Biochimica et Biophysica Acta BBA Biomembranes.* 2016;1858:2231–44.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.