Targeting of interleukin-10 receptor by a potential human interleukin-10 peptide efficiently blocks interleukin-10 pathway-dependent cell proliferation

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ABSTRACT

Objective: Human interleukin-10 (IL-10) is a dimeric and pleiotropic cytokine that plays a crucial role in cellular immunoregulatory responses. As IL-10 binds to its receptors, IL-10Ra and IL-10Rb, it will suppress or induce the downstream cellular immune responses to protect from diseases. Materials and Methods: In this study, a potential peptide derived from IL-10 based on molecular docking and structural analysis was designed and validated by a series of cell assays to block IL-10 binding to receptor IL-10Ra for the inhibition of cell growth. Results: The simulation results indicate that the designed peptide IL10NM25 bound to receptor IL-10Ra is dominated by electrostatic interactions, whereas van der Waals (VDW) and hydrophobic interactions are minor. The cell experiments showed that IL10NM25 specifically binds to receptor IL-10Ra on the cell surface of two B-lineage cell lines, B lymphoma derived (BJAB), and lymphoblastoid cell line, whereas the mutant and scramble peptides are not able to suppress the binding of IL-10 to receptor IL-10Ra, consistent with the molecular simulation predictions. Conclusion: This study demonstrates that structure-based peptide design can be effective in the development of peptide drug discovery.

KEYWORDS: Cytokine, Interleukin-10, Molecular docking, Peptide design

INTRODUCTION

Human interleukin-10 (IL-10) is the main member of the IL-10 cytokine family which consists of IL-19, IL-20, IL-22, IL-24, and IL-26 [1]. IL-10 is a pleiotropic cytokine whose expression can be prevalently found in many cell types, such as Th2 cells, macrophages, dendritic cells, B-cells, and various subsets of CD4+ and CD8+ T-cells [2]. IL-10 can suppress or activate cellular immune responses to protect the host against invading pathogens; therefore, the dual effects of IL-10 in both immunosuppression and innate immunity hamper the studies on its precise role in cancer development. The IL-10’s biological role in cancer likely depends on the cell types or restricted microenvironments; thus, developing both IL-10 pathway-specific agonists and antagonists is considered as potential IL-10 targeting strategy in anti-cancer therapy.

IL-10 is a dimeric cytokine that signals through a tetrameric transmembrane receptor complex, consisting of two IL-10Ra and two IL-10Rb proteins. IL-10 biological activity requires the sequential assembly of these two surface receptors (IL-10Ra and IL-10Rb) [3]. IL-10 initially binds to IL-10Ra with high affinity forming IL-10/IL-10Ra complex, and the intermediate complex is sequentially recognized by IL-10Rb with low affinity to form a IL-10/IL-10Ra/IL-10Rb ternary complex, which activates intracellular (IC) JAK family tyrosine kinases, and subsequently, IC signaling pathways, leading to cellular responses [4,5]. Previous studies have reported that overexpression of IL-10 promotes tumor development in certain lymphomas and melanomas by suppressing the antitumor immune response [6,7]. Nevertheless, recent advances in comparative database analyses reveal that serum IL-10 levels have been shown as a biomarker for predicting prognostic outcome of several types of human malignancies [8-10]. As IL-10 has been implicated in promoting cell growth and differentiation of activated human B lymphocytes [11,12], suggesting B-lineage cell-associated cancers could exploit the above biological feature to assist in carcinogenesis. In the past decades, peptide ligands have been utilized therapeutically as agonists or antagonists in several
diseases, such as metabolic diseases and oncology [13]. The attractive features of peptide drugs compared with small compounds and antibodies include high selectivity, low cost, low cytotoxicity, and immune response [13,14]. Our previous studies have shown the possibility that structure-based peptides can be a therapeutic strategy for anti-inflammation [15,16]. Therefore, in this study, we designed a specific peptide derived from IL-10 according to the resolved IL-10/IL-10Ra complex structure [17] using molecular docking and structural analysis to evaluate its specific binding to cell surface IL-10Ra and effects on cell growth of two B neoplastic cells. Our results suggested that the designed peptide IL10NM25 can specifically bind to IL-10Ra and effectively suppress the cell proliferation. The development of new anti-cancer therapies using peptide-based ligands has much potential in the future pharmaceutical medicine.

MATERIALS AND METHODS

Molecular docking of the designed peptide to interleukin-10Ra receptor

To predict the preferable binding sites between the designed peptides and the extracellular domain of receptor IL-10Ra. The docking module of Molecular Operating Environment software package (MOE2018.01 (Chemical Computing Group, Montreal, Canada)) (http://www.chemcomp.com) was used to perform the molecular docking and structural analysis.

Peptide synthesis

The designed and mutant peptides were determined for experimental confirmation based on the in silico analysis and molecular docking of this study. These peptides were chemically synthesized by the MS (MISSION BIOTECH, Taiwan) with a solid-phase methodology. The sequences of peptides used in this study were in the following,

IL10NM25: 21NMLRDLRDAFSRVKTFQMKDQLD N

mIL10NM25: 21NMLADLADASFVKTFQMKDQLD N

CF25: 1CPLNGSTVYGHRLHCLSCGTMVFK N

Cell lines, cell culture, lentivirus shRNA vectors, and cell viability assays

BJAB is a B-lymphoma cell line [18]. The EBV transformed lymphoblastoid cell line (LCL) has been established in the laboratory [19]. The above cell materials were cultured in RPMI1640 medium (Gibco BRL) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. The lentivirus-based shRNA vectors for human IL-10 were purchased from the National RNAi Core Facility, Academia Sinica Taiwan. For virus production, ~5 × 10^6 cells in a 10-cm cultured dish were transfected with lentiviral DNA derived from IL-10 according to the resolved IL-10/IL-10Ra complex structure [17] using molecular docking and structural analysis. Therefore, in this study, we designed a specific peptide derived from IL-10 according to the resolved IL-10/IL-10Ra complex structure [17] using molecular docking and structural analysis to evaluate its specific binding to cell surface IL-10Ra and effects on cell growth of two B neoplastic cells. Our results suggested that the designed peptide IL10NM25 can specifically bind to IL-10Ra and effectively suppress the cell proliferation. The development of new anti-cancer therapies using peptide-based ligands has much potential in the future pharmaceutical medicine.

Antibodies, immunostaining, enzyme-linked immunosorbent assay, and immunofluorescence flow cytometry

The expressing patterns of IL-10Ra or IL-10Rb in each cell line were analyzed by flow cytometry analysis. The antibodies for immunostaining are: Human IL-10Ra (R and D Systems, Cat: MAB274, 1:200) and Human IL-10Rb (R and D Systems, Cat: MAB874, 1:200). Fluorescein isothiocyanate (FITC) AffiniPure Goat Anti-Mouse IgG (H + L) (Jackson ImmunoResearch, #115-095-003, 1:100) was used to locate the proteins of interest by producing fluorescent images. Briefly, cells were rinsed three times and collected in 1X phosphate-buffered saline (PBS) with the necessary antibody. Cell staining was performed on ice for 30 min (min), and then, the secondary antibody was used following a PBS wash procedure. Cells were treated with the IC Fixation Buffer for 10 min (ThermoFisher Scientific) prior to performing immunofluorescence flow cytometry on Guava easyCyte HT (Millipore). The amount of IL-10 in cultured medium was quantitated using an IL-10-specific enzyme-linked immunosorbent assay (ELISA) kit (eBiosciences).

Fluorescein isothiocyanate-conjugated peptides preparation and labeling assays

Three FITC-conjugated synthetic peptides were included in this study. FITC-CF25 (Mission Biotech, Peptide ID: 992013) is a nonspecific peptide (negative control). FITC-IL10NM25 (Mission Biotech, Peptide ID: 992012) is a wild-type versus a corresponding mutant FITC-mIL10NM25 (Mission Biotech, Peptide ID: 992011). Each fluorochrome-conjugated peptide was prepared as 1 mg/mL in different solvents, including dimethyl sulfoxide (DMSO), PBS, deionized water, and cultured medium. Cells were rinsed three times and collected in 1X PBS with 0–50 µg/mL of each peptide. Samples were incubated at room temperature for 30 min and washed with PBS for three times before the immunofluorescence flow cytometry assay was performed. The positive staining cells were determined as percentage of a total.

For antibody-mediated competition assays, cells were pre-treated with the indicated amount of IL-10Ra/or IL-10Rb antibody or control IgG at room temperature for 30 min. Cells were then incubated with 30 µg/mL of each FITC-conjugated peptide for another 30 min after a PBS wash procedure. FITC signal was then quantified by flow cytometry soon as the free FITC peptides were removed by the PBS wash.

Statistical analysis

All the quantitative data were obtained after a procedure of comparative analysis. The statistical analysis was performed using Student’s t-test, and the statistical data were formulated as the mean ± standard deviation. *P < 0.05 represents the observed phenotype is significant to the compared reference, whereas †P > 0.05 means no difference was observed.
RESULTS

Surface charge and hydrophobicity distributions of the binding interface

The previous resolved IL-10/IL-10Ra complex structure showed that the N-terminal region of IL-10 binds to some loops and turns of the extracellular domain of IL-10Ra (PDB code: 1Y6K) [17]. The surface charge distribution of the binding interface between IL-10 and IL-10Ra indicated that electrostatic interactions might dominate the IL-10 binding to the extracellular domain of IL-10Ra. The binding region of IL-10 is more positively charged. The hydrophobicity map also indicated that the binding region between IL-10 and IL-10Ra is more hydrophilic [Figure 1]. From the surface charge distribution and hydrophobicity map analyses, we found that electrostatic interactions may play a crucial role in the binding of IL-10 to IL-10Ra, whereas VDW and hydrophobic interactions are minor.

Molecular docking of the designed peptides to interleukin-10Ra receptor

Based on the in silico analysis of the binding interface of IL-10/IL-10Ra complex structure, a potential peptide (named IL10NM25) was determined to inhibit IL-10 binding to receptor IL-10Ra. The designed peptide IL10NM25 with 25 amino acids derived from human cytokine IL-10 showed a helical conformation. The preferable pose of IL10NM25 redocked to IL-10Ra was almost superposed to the original complex structure with a quite low root-mean-square deviation value (0.101 Å) [Figure 2a]. Electrostatic interactions dominated the designed peptide bound to receptor IL-10Ra, which positively charged residues of the peptide (R24, R27, and K34) interact with the negatively charged residues (D100 and E101) and the hydrophilic residues with negative electric field (S190 and S192) of the receptor IL-10Ra [Figure 2a].

The designed interleukin-10 peptide efficiently targets to the cell surface of B-lineage cells

According to the molecular simulations, a peptide (IL10NM25) derived from IL-10 was theoretically predicted as the preferable candidate to compete with IL-10
for binding to IL-10Ra receptor. We also designed a mutant peptide (mIL10NM25) with three positively charged residues (R24, R27, and R32) mutated to alanine in the context of the same IL-10 peptide. Moreover, a scramble peptide CF25 was also designed for comparison. IL-10 is widely expressed in several types of lymphocytic cells; thus, many lymphocytic cells could be the ideal materials for IL-10 Ra blocking assays. In this study, we used two B-lineage cell lines, BJAB and LCL, to perform the peptide IL10NM25-mediated IL-10Ra targeting assays. The IL-10 receptor is a tetrameric complex, composed of two Ra and two Rb subunits. The expression pattern of each subunit on the cell surface area was determined by flow cytometry. Apart from showing both IL-10Ra and Rb were expressed on the cell surface, the expressed intensity of IL-10Ra is relatively higher than IL-10Rb among populations of two cell lines [Figure 3a and b]. The peptide IL10NM25 was prepared as 1 mg/mL in different solvents, including DMSO, H2O, PBS, and cultured medium, respectively. The peptide IL10NM25 was labeled with FITC to facilitate the flow cytometry analysis to be carried out properly. In BJAB cells, peptide FITC-IL10NM25 in the solvents with the above order target to 55%, 85%, 80%, and 75% of the population, whereas the same groups of peptide target to 50%, 70%, 85%, and 90% of LCL [Figure 3c and d]. To facilitate the manipulation of cell culture, 0–50 µg/mL of peptide FITC-IL10NM25 was prepared in cultured medium and used to assay for their targeting efficacy. Peptide FITC-IL10NM25 produced 18%–90% targeting effects in BJAB cells while eliciting 10%–90% targeting efficacy in LCL [Figure 3e and f].

The designed interleukin-10 peptide specifically targets to receptor interleukin-10Ra

Although the designed peptide IL10NM25 successfully targets to the cell surface of two testing cell lines, it remains unclear whether it hits to the correct target as IL-10Ra is expected as the target site. We next performed an antibody-mediated competition assay to confirm the designed peptide FITC-IL10NM25 indeed targets to IL-10Ra specifically. The selected cells were treated with each peptide supplemented with antibody for IL-10Ra, Rb, or IgG control at a final concentration 2.5, 5, or 10 µg/mL. In the control group (IgG treated), the peptide FITC-IL10NM25 exhibited 80% targeting efficacy versus ~10% by the scramble peptide FITC-CF25 in both BJAB and LCL [Figure 4a and b]. Two set of similar results were obtained from the competition assays. IL-10Ra antibody at 2.5, 5, and 10 µg/mL interfered 30%, 35%, and 60% FITC-IL10NM25 targeting effects, whereas IL-10Rb antibody or IgG control at the same above preparations had no blocking activity compared to IL-10Ra antibody.

The interleukin-10 pathway is required for cell proliferation of B-lineage cell lines

Prior to validating the peptide-mediated IL-10 blocking effects on cell proliferation, we sought to demonstrate that the IL-10 pathway-dependent cell proliferation indeed existed in two B-lineage cell lines, BJAB and LCL cells. Each cell line was initiated in a fresh medium after a PBS wash procedure, and the amounts of IL-10 in the medium were determined by performing an ELISA assay after 24 h of incubation. The results indicated that approximately 150 pg/mL IL-10 was secreted into the cultured medium from either BJAB or LCL compared to the control medium [Figure 5a]. The specific monoclonal antibodies for IL-10Ra, Rb, or both were used to perform a neutralization assay. With a single or double treatment of the IL-10 receptor antibodies caused an 80% and 70% reduction in cell proliferation of LCL versus BJAB after 96 h [Figure 5b]. The IL-10 recombinant protein (rIL-10) at the final concentration of 0, 0.5, 1, and 10 ng/mL was next used to treat the selected cells for 24 h. The stimulating effects of rIL-10 on cell proliferation appeared in a dose-dependent manner, which caused a 15%–60% increase from 0.5 to 10 ng/mL compared to the control group [Figure 5c]. In the same experimental design, adding of IL10NM25 peptide at 30 µg/mL caused a 50% blocking effect on rIL-10-stimulated cell proliferation by 24 h. The activation of IL-10 signaling pathway was validated by the expression levels of its downstream indicator, phosphorylated STAT3 (pSTAT3), in IL-10 shRNA (shIL-10) knockdown BJAB or LCL cells. A >90% of IL-10 depletion at the protein levels were observed in two shIL-10 transduced cell lines compared to each scramble shRNA (shScr) transduced group (control) [Figure 5d]. Consequently, it led to a ~70% reduction of pSTAT3 compared to an unaltered expressed level of total amount of STAT3 in each case [Figure 5e]. Similar to the antibody-mediated blocking experiments described elsewhere, IL-10 depletion by shRNA resulted in an 80% reduction of cell viability in each cell line [Figure 5f]. Apparently, our data support that IL-10 is crucially implicated in cell proliferation of two B-lineage cell lines used in this study.

The designed interleukin-10 peptide exhibits biological impacts to debilitate cell growth

Cumulative evidence has disclosed variable effects of IL-10 on cell proliferation of human B-lineage cells [20]. A complete set of the synthetic peptides, FITC-CF25, FITC-IL10NM25, and FITC-mIL10NM25, were then used to verify their effects on cell proliferation of two selected cell lines. The peptide FITC-IL10NM25 exhibited >90% targeting efficacy in two cell lines, whereas FITC-mIL10NM25 only retained 30%–40% of the effect [Figure 6a and b]. FITC-CF25 only produced a noise signal by <10% targeting efficacy. The selected cells treated with each peptide were monitored for cell proliferation for 96 h. At the endpoint, the peptide FITC-IL10NM25 almost entirely blocked the cell proliferation of LCL versus debilitating 80% cell growth of BJAB compared to FITC-mIL10NM25 or FITC-CF25 [Figure 6c and d]. Of importance, we showed that IL10NM25 caused a 55% and a 61% reduction of pSTAT3, whereas mIL10NM25 barely resulted in a reduction of pSTAT3 by 15% and 9% in LCL versus BJAB cells [Figure 6e]. Our data clearly indicate the specific IL-10Ra targeting effects could lead to a truly biological impact on cell proliferation, as they efficiently impaired IL-10 signaling pathway.

Discussion

Up to now, the complex structure for IL-10 bound to complete IL-10Ra and IL-10Rb is still not resolved, only the structure for IL-10 bound to extracellular domain of
Figure 3: Interleukin-10 peptides efficiently target to the cell surface of B-lineages cells. (a) Both BJAB and (b) lymphoblastoid cell line cells were subjected for an immunostaining procedure using antibodies for interleukin-10Ra and interleukin-10Rb, respectively. The fluorescein isothiocyanate-conjugated secondary antibody was used to produce fluorescent signals in those interleukin-10Ra- or interleukin-10Rb-positive cells. The expressing pattern of each interleukin-10 receptor was analyzed by flow cytometry. (c) The fluorescein isothiocyanate-conjugated interleukin-10 peptides (fluorescein isothiocyanate-interleukin-10NM25) were dissolved in dimethyl sulfoxide, ddH$_2$O, phosphate-buffered saline, or culture medium. BJAB and (d) lymphoblastoid cell line cells were treated with 30 $\mu$g/mL of the above peptides, respectively. The binding efficiency of each interleukin-10 peptide to the cells was quantified by flow cytometry. The peptides fluorescein isothiocyanate-interleukin-10NM25-labeled cells were determined as the percentage of a total. For here and the following experiments, cell samples without a peptide treatment were used as negative control. (e) BJAB or lymphoblastoid cell line cells (f) treated with the peptide fluorescein isothiocyanate-IL10NM25 at a concentration of 0, 0.1, 0.5, 1, 5, 10, 20, and 50 $\mu$g/mL, respectively. The distribution of peptide labeling cells was quantified by flow cytometry followed the procedure as described elsewhere.
IL-10Ra is available, which is supported to design a potential peptide derived from IL-10 to suppress the binding of IL-10 to receptor IL-10Ra. The docking score of docking program of MOE software for peptide IL10NM25 was 65.10, whereas that for the mutant peptide mIL10NM25 was −54.56, indicating that the three mutant residues (R24A, R27A, and R32A) of mIL10NM25 declined the binding affinity to receptor IL-10Ra. The interaction maps for the two peptides with nearby receptor indicated that more residues of IL10NM25 interact with IL-10Ra (R91, D100, A189, S190, S192, and N193) than that of mIL10NM25 (R191 and S192), consistent with the docking results [Figure 2b and c].

In cellular experiments, the synthetic peptide IL10NM25 specifically bounds to the receptor IL-10Ra on the cell surface of two B-lineage cell lines, BJAB and LCL [Figures 3 and 4] verified the molecular docking results. Moreover, the mutant peptide mIL10NM25 reduced the electrostatic interactions to receptor IL-10Ra; imply it not preferable for binding to receptor. The comparison of three synthetic peptides bound to receptor IL-10Ra in cell assays indicated that IL10NM25 can entirely blocking the binding to IL-10Ra and inhibit the cell proliferation, whereas the mutant peptide mIL10NM25 retained 30%–40% of the binding effect and the scramble peptide CF25 rather produced binding efficacy <10% [Figure 5]. The cellular assays confirmed the molecular docking prediction and specificity of IL10NM25 binding to receptor IL-10Ra.
Conclusion

In this study, we designed a potential peptide IL10NM25-derived human cytokine IL-10 based on the resolved IL-10/IL-10Ra complex structure to inhibit IL-10 binding to its receptor IL-10Ra. Structural analysis and molecular docking indicate that electrostatic interactions dominated the binding of peptide IL10NM25 to receptor IL-10Ra and three key residues (R24, R27, and R32) were also found to play an crucial role in the binding to IL-10Ra. A series of cell assays confirmed the docking predictions and designed peptide IL10NM25 effectively inhibit the cell proliferation of selected cell lines by blocking the binding of IL-10 to IL-10 receptor. Structure-based in silico analysis is a powerful tool which can be applied to design a preferable peptide, or small compounds to serve as therapeutic agents to treat diseases in the near future.

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Figure 6: Interleukin-10 peptides exhibit potent inhibitory effects on cell growth. (a) BJAB or (b) lymphoblastoid cell line cells were treated with 50 µg/mL fluorescein isothiocyanate-CF25, fluorescein isothiocyanate-IL10, and fluorescein isothiocyanate-mutIL10 peptides at RT for 30 min before subjecting to perform flow cytometry analysis. The fluorescein isothiocyanate-positive cells were calculated as the percentage of a total ± standard deviation The none-peptide-treated group was used as negative control. The fluorescein isothiocyanate-mut interleukin-10 versus fluorescein isothiocyanate-interleukin-10-labeling efficiency was compared with *P < 0.05. (c) BJAB or (d) lymphoblastoid cell line cells were treated with the indicated peptides at 50 µg for 96 h. Cell viability assays were performed every 24 h using RealTime-Glo™ MT assay kit, and data were determined as the RLU. (e) 5 × 10^6 BJAB or lymphoblastoid cell line cells were treated with the indicated peptides at 50 µg/mL for 48 h. Cell lysates derived from each sample were immune blotted for pSTAT3, STAT3 and actin control. The images are shown. Lymphoblastoid cell line (left) versus BJAB (right).

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Fickenscher H, Hör S, Küppers H, Knappe A, Wittmann S, Sticht H. The interleukin-10 family of cytokines. Trends Immunol 2002;23:89-96.
2. Wilson EB, Brooks DG. The role of IL-10 in regulating immunity to persistent viral infections. Curr Top Microbiol Immunol 2011;350:39-65.
3. Kotenko SV, Krause CD, Izotova LS, Pollack BP, Wu W, Pestka S. Identification and functional characterization of a second chain of the interleukin-10 receptor complex. EMBO J 1997;16:5894-903.
4. Finbloom DS, Winestock KD. IL-10 induces the tyrosine phosphorylation
of tyk2 and Jak1 and the differential assembly of STAT1 alpha and STAT3 complexes in human T cells and monocytes. J Immunol 1995;155:1079-90.

5. Yoon SI, Jones BC, Logsdon NJ, Harris BD, Kuruganti S, Walter MR. Epstein-Barr virus IL-10 engages IL-10R1 by a two-step mechanism leading to altered signaling properties. J Biol Chem 2012;287:26586-95.

6. Boulland ML, Meignin V, Leroy-Viard K, Copie-Bergman C, Brière J, Touitou R, et al. Human interleukin-10 expression in T/natural killer-cell lymphomas: Association with anaplastic large cell lymphomas and nasal natural killer-cell lymphomas. Am J Pathol 1998;153:1229-37.

7. Huang S, Ullrich SE, Bar-Eli M. Regulation of tumor growth and metastasis by interleukin-10: The melanoma experience. J Interferon Cytokine Res 1999;19:697-703.

8. Zhao S, Wu D, Wu P, Wang Z, Huang J. Serum IL-10 predicts worse outcome in cancer patients: A meta-analysis. PLoS One 2015;10:e0139598.

9. de Oliveira JF, Wiener CD, Jansen K, Portela LV, Souza LD, et al. Serum levels of interleukins IL-6 and IL-10 in individuals with posttraumatic stress disorder in a population-based sample. Psychiatry Res 2018;260:111-5.

10. Sheikhpour E, Noorbakhsh P, Foroughi E, Farahnak S, Nasiri R, Neamatzadeh H. A survey on the role of interleukin-10 in breast cancer: A narrative. Rep Biochem Mol Biol 2018;7:30-7.

11. Rouset F, Garcia E, Defrance T, Péronne C, Vezzio N, Hsa DH, et al. Interleukin 10 is a potent growth and differentiation factor for activated human B lymphocytes. Proc Natl Acad Sci U S A 1992;89:1890-3.

12. Béguelin W, Sawh S, Chambwe N, Chan FC, Jiang Y, Choo JW, et al. IL10 receptor is a novel therapeutic target in DLBCLs. Leukemia 2015;29:1684-94.

13. Fosgerau K, Hoffmann T. Peptide therapeutics: Current status and future directions. Drug Discov Today 2015;20:122-8.

14. Vanhee P, van der Sloot AM, Verschueren E, Serrano L, Rousseau F, Schymkowitz J. Computational design of peptide ligands. Trends Biotechnol 2011;29:231-9.

15. Jiang SJ, Liou JW, Chang CC, Chung Y, Lin LF, Hsu HJ. Peptides derived from CXCL8 based on in silico analysis inhibit CXCL8 interactions with its receptor CXCR1. Sci Rep 2015;5:18638.

16. Jiang SJ, Tsai PI, Peng SY, Chang CC, Chung Y, Tsao HH, et al. A potential peptide derived from cytokine receptors can bind proinflammatory cytokines as a therapeutic strategy for anti-inflammation. Sci Rep 2019;9:2317.

17. Yoon SI, Jones BC, Logsdon NJ, Walter MR. Same structure, different function crystal structure of the Epstein-Barr virus IL-10 bound to the soluble IL-10R1 chain. Structure 2005;13:551-64.

18. Takimoto T, Sato H, Ogura H. Primary EBV infection of human umbilical cord lymphocytes and EBV genome-negative lymphoblastoid cell lines (BJAB and Ramos). Auris Nasus Larynx 1986;13:199-205.

19. Shen CL, Liu CD, You RI, Ching YH, Liang J, Ke L, et al. Ribosome Protein L4 is essential for epstein-barr virus nuclear antigen 1 function. Proc Natl Acad Sci U S A 2016;113:2229-34.

20. Itoh K, Hirohata S. The role of IL-10 in human B cell activation, proliferation, and differentiation. J Immunol 1995;154:4341-50.