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The rational designed antagonist derived from the complex structure of interleukin-6 and its receptor affectively blocking interleukin-6 might be a promising treatment in multiple myeloma

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Abstract

Human interleukin-6 is involved in the maintenance and progression of several diseases such as multiple myeloma (MM), rheumatoid arthritis, or osteoporosis. Our previous work demonstrated that an interleukin-6 antagonist peptide (named PT) possessed potential bioactivity to antagonize the function of hIL-6 and could efficiently induce the growth arrest and apoptosis of XG-7 and M1 cells in a dose-dependent manner. In this study, the theoretical interaction of the peptide PT with its receptor was analyzed further more with molecular docking and molecular dynamics methods. The theoretical studies showed that PT possessed very high affinity to interleukin-6R and offered a practical means of imposing long-term blockade of interleukin-6 activity in vivo. According to the theoretical results, the biological evaluation of PT was researched on two different cells models with more sensitive approaches: (1) The antagonist activity of PT was studied on the interleukin-6 dependent MM cells (XG-7) cultured with interleukin-6. In the other interleukin-6 dependent MM cells (SKO-007), they survived themselves by auto/paracrine without the exogenous interleukin-6, and also could be antagonized by PT. The therapeutic value of PT only limited on the interleukin-6 dependent category in MM. (2) Myeloid leukemia M1 cells were induced for growth arrest and apoptosis in response to interleukin-6. The results supported our previous findings and showed that PT could be evaluated by protecting the cells from interleukin-6 induced apoptosis. In conclusion, PT could induce interleukin-6-dependent XG-7 and SKO-007 cells to apoptosis while inhibit interleukin-6-stimulated apoptosis in M1 cells.

Keywords: Interleukin-6; Rational design; Apoptosis; Interleukin-6 receptor; 3-D complex structure

1. Introduction

Human interleukin-6 (IL-6) was a typical multifunctional cytokine involved in the regulation of proliferation, differentiation of hematopoietic cells and pathogenesis of many diseases [1]. It was a member of the IL-6-type cytokines subfamily comprising IL-6, IL-11, ciliary neurotrophic factor, oncostatin M, cardiotrophin-1, and cardiotrophin-like cytokine [2]. IL-6 acted through a surface receptor, belonging to the hematopoietin receptor superfamily, composed of two chains: an 80 kDa ligand binding protein (gp80, IL-6R) and a 130 kDa protein (gp130) [3,4]. gp130 had no intrinsic binding properties with IL-6, but required for the generation of high affinity to form proper hexametric complex. Although IL-6 did not function as a growth factor for normal B-cells, proliferating plasma blasts, it was involved in the origin of some benign and malignant plasma cell expansions [5]. Anderson [6] considered that IL-6 was involved in the multiple myeloma (MM) pathogenesis and able to stimulate the growth of MM cells from patients. In the clinic, high levels of circulating IL-6 reflected a worse course of the disease [7]. Experiment had demonstrated that MM cells were frequently able to produce IL-6 in a para/autocrine fashion and sometimes expressed the functional IL-6 receptor [8].

Anti-cytokine therapies were mostly aimed at the inhibition of IL-6 and its receptor that was responsible for the comeback of MM disease [9]. IL-6 monoclonal antibodies (mAbs) had been proposed as therapeutic tools for IL-6-related pathologies, since it formed a complex with IL-6 and resulted in reduced clearance of the cytokine [10]. However, antibodies could inhibit IL-6 only partially in the form of circulating complexes

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conducting effectively in a minority of MM patients [11]. The limitation consisted not only in allowing for administration of high and repeated doses of mAbs, but also potentially immunogenic in the long-term efficacy of these treatments. Therefore, we tried to investigate a new selective antagonist on the basis of extensive investigation of the IL-6/IL-6 receptor complex interaction [12]. As an alternative approach to block the actions of IL-6, the use of peptide antagonists rather than mAbs, was an innovative and promising approach for the treatment of cytokine-mediated disease.

The three dimensional complex structure of IL-6, IL-6R and gp130 has been determined by X-ray crystallography [12]. Experiment has argument that three distinct sites constitute the contact points of IL-6 with its receptors. The site I binding epitope of IL-6 is localized to the A and D helices and interacts with IL-6R formed the initial IL-6/IL-6R binary complex. The site IIa is between the IL-6 A and C helical faces and the "elbow" region at the boundary between the D2 and D3 domains of gp130 CHR, and site IIb is between the IL-6R D3 domain and the gp130 D3 domain. The site III is a broad and discontinuous interface between IL-6 and gp130 where the tip of the IL-6 four-helix bundle (AB loop and N-terminal region of D helix) abuts into the bottom sheet of the D1 domain of gp130 and includes residues mapped to the site III epitope of IL-6. The formation of the site III interaction is enabled by the curvature of the IL-6 B and D helical axes that form site I [12,13].

In our previous work, a highly specific antagonist peptide (named as PT) was designed on the basis of theoretical models constructed via computer-aided methods and extensive investigation of IL-6/IL-6R complex interaction. PT possessed potential bioactivity to antagonize the function of hIL-6 [14,15]. In this report, the theoretical interaction of PT with IL-6R was studies further. PT acted through their active chemical groups to bind IL-6R in competition with their natural ligand identified as the most potent super-antagonist. In addition, PT induced the apoptosis of XG-7 cells was studied further by electronic microscopy and FACS. The results were in consistent with our previous report [15]. PT was also able to induce the IL-6-dependented MM cell line SKO-007 which supported their own growth loop by auto/paracrine minute amounts of IL-6 to undergo a rapid growth arrest and apoptosis [16,17]. Murine myeloid leukemia cells (M1) underwent a cell cycle arrest and terminal differentiation following IL-6 stimulation [18,19]. In this report, using M1 myeloblastic leukemia cells as a model, IL-6 did not rather sustain cells growth than induce cells apoptosis to evaluate IL-6 antagonist that had never been reported. This cells model different from cytokine-depended cells was another inspective method to detect the interaction of relative cytokine/cytokine receptor.

2. Materials and methods

2.1. Theoretical evaluation of PT

Based on the residues sequences of de novo designed antagonist peptide PT [14,15], the secondary structure of PT was predicted using GOR IV [20] method derived from http://www.expasy.ch. According to the predicted secondary structure and Ramachandran et al.'s map [21] to limit the torsion angles (ϕ, ψ) of each residues due to the interactions of the side-chain with its backbone, the 3-D structure of PT was constructed as original model. Moreover, minimizations and short molecular dynamics (MD) simulations (1000 ps) were performed by means of the program Discover_3 dealing with CVFF and AMBER forcefield. After structural optimization of PT, the positions and orientations of the side chains of the key residues were checked.

With molecular docking method, the 3-D complex structure of PT and IL-6R was constructed using the modeled structure of PT and crystal structure of IL-6R (PDB code: 1N26) [22]. In order to obtain the favorable complex structure, solvent effect was considered during the 3-D structure was optimized. In this study, our MD system was performed with 5-Å water layers for the structure. The system was heated to 300 K with 5 °C rise per 5000 steps by randomly assigning velocities from the Gaussian distribution. After heating, the system was allowed to equilibrate for 50-ps. And then, the system was subjected to 500-ps MD simulation at constant temperature (300 K). The results were collected every 5 ps from the 1000-ps trajectory. The structures, averaged over the 2000-ps of the equilibrated time period of each MD simulation, were then minimized.

2.2. Cell culture

Human MM cell line SKO-007, XG-7 (a kind gift from Professor Xue-guang Zhang of Suzhou University, China); murine myeloid leukemia cell line M1 (ATCC, USA) and mouse fibrosarcoma L929 cells were grown in RPMI1640 supplemented with 10% fetal bovine serum, penicillin–streptomycin (50 IU/ml and 50 g/ml, respectively), and maintained at 37 °C in 5% CO_2 atmosphere. In XG-7 cells, recombinant IL-6 was added to the final concentration of 10 ng/ml [23–25].

2.3. PT treatment

The peptide (Lot NO: 10045554) was synthesized at Genemed Synthesis (CA, USA). XG-7, SKO-007 and L929 cells were washed three times with RPMI 1640 medium and seeded at 5×10^4 cells per ml in the above-described conditions for 12 h with 1640 + 2% FBS. M1 cells seeded at 2×10^4 cells per ml with recombinant IL-6 to the final concentration of 100 ng/ml. PT was added at the concentration of 0.04, 0.4, and 4 μg/ml (after 2 h incubation, the IL-6 was restored at 10 ng/ml only in XG-7 cells) for 4 days. The experiment was divided into six groups: (1) Normal control group (normal cells, XG-7 cells cultured with 10 ng/ml IL-6,). (2–4) Different concentrations of PT (0.04, 0.4, 4 μg/ml PT. XG-7 cells re-added with 10 ng/ml IL-6; L929 cells with 2.5 μg/ml TNFα and 1 μg/ml actinomycin; M1 cells with 100 ng/ml IL-6). (5) NRP (4 μg/ml, non-related peptide) (15 amino acid peptide not related with PT. XG-7 re-added with 10 ng/ml IL-6; M1 cells with 100 ng/ml IL-6). (6) Positive group (XG-7 cells without