Computational Prediction for the Binding Affinity of Interleukins 3 and 5 and GM-CSF to Cell Surface Receptors on Human Eosinophils

Shahin Gavanji*, Hassan Mohabatkar2

1Young Researchers and Elite Club, Khorasgan Branch, Islamic Azad University, Khorasgan, Isfahan, Iran
2Department of Biotechnology, Faculty of Advanced Sciences and Technologies, University of Isfahan, Isfahan, Iran.

*Corresponding Author: shahin.gavanji@yahoo.com

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Abstract. Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a 14.477 kD glycoprotein comprising 144 amino acids residues. The respective encoding gene is located on chromosome 5 in human. This protein stimulates proliferation and differentiation of macrophages. N-terminally seventeen amino acid residues are serving as a signal peptide while, the rest of 127 amino acids, known to have therapeutics application, is termed Molgramostim. Previous studies have revealed a high affinity of this protein for binding to a heterodimer receptor on surface of the cell. The respective receptor includes a and β chains which the β chain is similar to interleukins 3 and 5 receptors. Due to this similarity, interleukins 3 and 5 are capable to compete with GM-CSF in binding to the shared receptor. In the present study, to evaluate the binding affinity of interleukins 3 and 5 and GM-CSF to the same receptor, a computational prediction study carried out using Modeller, Hex and Molegro softwares. According to the results, interleukin 3 with -517.09 kJ/mole, interleukin 5 with -538.05 kJ/mole and GM-CSF with -606.17 kJ/mole energy could bind to the a and β chains of receptor. In the next step the two chains of the receptor were separated and the affinity of each protein to both chains was studied. Based on the results the binding affinity of all three considered proteins to a chain of the protein was weaker than the binding to β chain. The binding energy of interleukin 3, interleukin 5 and GM-CSF to β chain of receptors was -620.37 kJ/mole, -663.80 kJ/mole and -696.07 kJ/mole respectively. According to the results, interleukin 3 and interleukin 5 strongly compete with GM-CSF in binding to cell surface receptors on human eosinophils.

Keywords: Docking, GM-CSF, Interleukin, Receptor.

1. INTRODUCTION

One of the most important cytokine is human granulocyte macrophage colony stimulating factor (hGMCSF) which is secreted by macrophages, T-lymphocyte cells, mast cells, endothelial cells and fibroblasts (Schwanke et al., 2009). This protein has molecular weight of 14.477 kD comprises 144 amino acids residues. Seventeen amino acid residues from the N-terminus of hGMCSF are serving as a signal peptide while, the rest of 127 amino acids are known as Molgramostim. This protein is also termed as CSF-2 and CSFa. hGM-CSF is an important therapeutic cytokine which is used in the treatment of myeloid leukemia, neutropenia and aplastic anemia diseases (Armitage, 1985). Moreover, hGM-CSF increases the effector functions of neutrophils, eosinophils and the antigen-presenting ability of monocytes (Vadas et al., 1983; Morrissey et al., 1987). Moreover, hGM-CSF might increase anti-tumour responses (Hill et al., 1993). Interleukins (ILs) are a group of soluble cytokines which are produced by macrophages (Clutterbuck et al., 1987). Interleukin 3 (IL-3) and 5 (IL-5) are produced by T cells and some of their activities included proliferation stimulation, differentiation and survival of myeloid hemopoietic and also regulate the hematopoiesis and inflammation (Kafert et al., 1999).

IL-3 binds to respective receptors at the cell surface to stimulate the proliferation of early multipotential progenitors (Metcalf and Nicola, 1995). Recombinant hIL-3 is widely applied for treatment of thrombocytopenia (Denzlinger et al., 1993). IL-5, is an important cytokine which is synthesized by activated T-helper 2 cells (Adachi et al., 1995). Understanding the regulatory mechanisms of this protein may be helpful to explore effective ways for the control of allergic diseases. It has been shown that in disease like asthma the amount of GM-CSF, IL-3, and IL-5 increased in the airways of patients. (Adachi et al., 1995). Additionally GM-CSF and IL-3 are so important in fighting with microbial pathogens.
Introduction and release of cytokines. Inflammation and infection. The cytokines consist of a ligand-specific α subunit (GM-α, IL-3Rα, and IL-5Rα) and a common β subunit (βC) (Hayashida et al., 1990). The α subunit is the main ligand-binding subunit (Robb et al., 1995; Murata et al., 1992) and the βC subunit converts the ligand-bound α subunit to a high affinity state and so it is important for signal transduction (Kitamura et al., 1991; Kitamura et al. 1991(b); Hayashida et al., 1990; Bagley et al., 1997). It is observed the three cytokines compete for induction of cell proliferation and survival through the same signal transduction pathway in eosinophil cells and leukemic cell line TF-1 (Adachi et al., 1995; Kitamura et al., 1991; Bagley et al., 1995; Murata et al., 1992; Ohnishi et al., 1993). The GM-CSF receptor was first defined using binding of radioactive GM-CSF to mouse and human bone marrow cells and cell lines (Park et al., 1986; Walker et al., 1985). The GM-CSF receptor α chain gene (CSF2RA) occurs in the pseudoautosomal region of the X and Y chromosomes at Xp22.32 in humans (Gough et al., 1990; Rappold et al., 1992). The common β chain gene (CSF2RB) is on chromosome 22q12.2-13.1 in humans (Shen et al., 1992). In humans the GM-CSF and IL-3 receptor β chains are closely linked (within 190 kb) in the pseudoautosomal regions of the X and Y chromosomes (Kremer et al., 1993).

The human α chain gene consists of 13 exons spanning about 44 kb (Nakagawa et al., 1994) as GM-CSF receptor is expressed in different types of cancer like acute myeloid leukemia, chronic myeloid leukemia, melanoma, certain breast cancer cell lines and the prostate (Baldwin et al., 1991; Baldwin et al., 1989) simultaneous inhibition of expression of the βc subunit of the receptors for GM-CSF, IL-3, and IL-5 receptors might be lead to specifically inhibiting growth and development of cancer cells. Most types of myeloid progenitors and mature monocytes, neutrophils, eosinophils, and dendritic cells have GM-CSF receptors. In this study we bound GM-CSF, IL-3, and IL-5 to their receptors and predicted the energy of affinity on the basis of kJ/mole. And also we observed the competition of GM-CSF, IL-3, and IL-5 in binding to Cell Surface Receptors on Human Eosinophils. We determined the best form of GM-CSF in order to bind to its receptor on Human Eosinophils.

2. MATERIALS AND METHODS:

As The first step, amino acid sequences of the human Granulocyte-macrophage colony-stimulating factor (GM-CSF) (Genbank ID: 1437), its receptor (Genbank ID: 1438 were), interleukin 3 (Genbank ID: 3562 ) and interleukin 5 (Genbank ID: 3567) and their receptor subunits (Genbank IDs: 1439 and 3568 for IL5-α and IL3-β receptors, 3563 IL3-α and 1439 IL5-β) were deduced from NCBI Entrez protein data base (www.ncbi.nlm.nih.gov). GM-CSF comprises 144 amino acid residues which 17 residues from its C-terminus are accounted as a signal peptide removable from the mature structure of protein. Due to the lack of crystal and three dimensional structure of IL3 and IL5 and their receptors in Protein Data Bank (PDB ) , we implemented online Server named PHYRE2 Protein Fold Recognition Server (www.sbg.bio.ic.uk/phyre2/). Furthermore, to predict of three dimensional structure of IL3 and IL5 and their receptors a comparative modeling was performed by utilizing SWISS-MODEL database (swissmodel.expasy.org/). As GM-CSF, IL3, IL5 and their receptors possess α and β chains, we designed the chain and β chain of the proteins separately.

2.1. Computational Molecular docking

Hex software calculates protein-ligand docking and binding affinity (kcal/mol). Assuming the ligand rigidity, it can bind to the pair subunit receptor by using three dimensional structures of molecules. Furthermore, docking and superposition program to use spherical polar Fourier (SPF) correlations to accelerate the calculations (Ritchie, 2003). Hex-6bwins downloaded on to the system and the molecular Interphase tool is opened. In this we measured the Binding affinity (kcal/mol) GM-CSF, IL3, IL5 toward their receptors on the basis of known experimental data. And also we observed the binding differences of the α chain and β chain. Finally we made a mutation in Glutamic acid 21 and we replaced it with Arginine by using Molegro softwares. As the result we could enhance Binding affinity (kcal/mol) of GM-CSF protein to α chain of GM-CSF receptor.

3. RESULTS AND DISCUSSIONS

According to table 2, molecular docking analyses of the human Granulocyte-macrophage colony-
stimulating factor (GM-CSF), interleukin 3 and interleukin 5 and their receptors showed that these proteins in silico condition bind to their receptors with close energy values. Prediction of interaction energies between ligand and receptor has been a major challenge for molecular docking. The PDB structures of (hGM-CSF) docked with its receptor with E – totals of -606.17 kJ/mole and in docking with α chain of GM-CSF receptor with E – totals of -340.34 kJ/mole and with β chain with E – totals of -696.07 kJ/mole. First we docked the IL3 and its receptor and the binding affinity was equal to -517.09. Then we docked IL3 with α chain of IL3 receptor which the energy was equal to -479.82 and with β chain with E – totals of -620.37 Binding affinity of interleukin 5 with its receptor was -538.05 And with α and β chain of its receptors was-539.46 and -663.80 respectively (Table 1). At last we made a mutation in Glutamic acid 21 and we replaced it with by using Molegro program. As the result we could enhance Binding affinity (kcal/mol) of GM-CSF protein to α chain of GM-CSF receptor.

Table1: protein- protein interactions

<table>
<thead>
<tr>
<th>No</th>
<th>Interactions</th>
<th>E – totals(kJ/mole)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>hGM-CSF and hGM-CSF receptor</td>
<td>-606.17</td>
</tr>
<tr>
<td>2</td>
<td>hGM-CSF and α chain of GM-CSF receptor</td>
<td>-340.34</td>
</tr>
<tr>
<td>3</td>
<td>hGM-CSF and β chain of GM-CSF receptor</td>
<td>-696.07</td>
</tr>
<tr>
<td>4</td>
<td>IL3 and its receptor</td>
<td>-517.09</td>
</tr>
<tr>
<td>5</td>
<td>IL3 with α chain of IL3 receptor</td>
<td>-479.82</td>
</tr>
<tr>
<td>6</td>
<td>IL3 with β chain of IL3 receptor</td>
<td>-620.37</td>
</tr>
<tr>
<td>7</td>
<td>Interleukin 5 with its receptor</td>
<td>-538.05</td>
</tr>
<tr>
<td>8</td>
<td>Interleukin5 with α chain of Interleukin5 receptor</td>
<td>-539.46</td>
</tr>
<tr>
<td>9</td>
<td>Interleukin5 with β chain of Interleukin5 receptor</td>
<td>-663.80</td>
</tr>
</tbody>
</table>

Table2: Summary of the binding and competition of IL-5, IL-3 and GM-CSF

<table>
<thead>
<tr>
<th>Competition by</th>
<th>IL 5</th>
<th>IL 3</th>
<th>GM-CSF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eosinophils</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interleukin 5</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>GM-CSF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interleukin 3</td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Receptors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monocytes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interleukin 5</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Interleukin 3</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>GM-CSF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Receptors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutrophils</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interleukin 5</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Interleukin 3</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GM-CSF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Receptors</td>
<td></td>
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</tr>
</tbody>
</table>

Data demonstrated that human eosinophil cells express high affinity receptors for IL-5 and IL-3 while, GM-CSF compete with IL-5 in binding to those receptors. The competition between IL-5, IL-3, and GM-CSF on the surface of eosinophils is important to understand the common activation properties of these cytokines. Recent studies on eosinophil functions have shown that IL-5 as well as IL-3 and GM-CSF share the similar effects on: enhancement of eosinophil superoxide production (Gregory et al., 2003), antibody dependent killing of tumor cells (Paul et al., 1997) and helminthes (Behm and Ovington, 2000), degranulation of the cell (Martinez-Moczygemba and Huston, 2001), survival ability of the cell in vitro (Zheng et al., 2002; Miike et al., 1999), conversion from a normodense to a hypodense phenotype, phagocytosis(Tai et al., 1991), and the biosynthesis of proteoglycans (Rothenberg et al., 1988). Interactions with a common receptor complex, implies one unifying mechanism. However, utilization of a common signal-transduction pathway downstream from the receptor remains to be explored. The second implication of the present findings is that the competition between IL-5, IL-3, and GM-CSF may serve to limit eosinophil stimulation, thus preventing the excessive release of oxygen products, leukotrienes, and granule contents of these cytokines with potentially harmful consequences to the host.


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(Lopez et al., 1992; Lopez et al., 1992b). The competition for I-IL-5 binding to eosinophils by 11.3 and GM-CSF was partial even when tested at high concentrations of competitors. This may be due to the association of some but not all IL-5 receptors with IL-3 and GM-CSF receptors or to the existence of eosinophil subpopulations expressing either IL-5-specific receptors, r IL-S-, IL-1-1, and GM-CSF-associated receptors. Morphological and functional differences have been noted in eosinophils generated in vitro and in vivo (Tai et al., 1991).

The physical nature of the competition between IL-5, IL-3 and GM-CSF is not yet known, but it may reflect the relatedness of these three eosinophil growth factors and their receptors. The relatedness between IL-5, IL-3, and GM-CSF extends from the structure and close localization of their genes on the long arm of chromosome 5 (van Leeuwen et al., 1989) to the structure of the mature polypeptides. The IL-5, IL-3, and GM-CSF molecules have conserved features, in particular an area of hydrophilic amino acids in the COOH terminus, and their tertiary structure is predicted to be highly conserved (Lin et al., 2000). It is clear that while in eosinophils the cross-reaction is most evident, in monocytes IL-3 and GM-CSF cross-compete (Kaushansky et al., 1992; Elliott et al., 1992) but IL-5 is unable to compete, and on neutrophils neither IL-5 nor IL-3 compete for GM-CSF binding. It is apparent therefore that for inhibition to occur the homologous receptor needs to be expressed (Table 2), and the unique pattern of competition between IL-5, IL-3, and GM-CSF is observed on eosinophils because these cells express all three receptors.

4. CONCLUSION

Different studies showed that various factors are involved in growth and differentiation of red and white blood cells. Cytokines secreted from immune cells like T lymphocyte and Macrophage play special role in differentiation of blood cells. Granulocyte Macrophage cloning stimulating factor (GM-CSF) is one of these factors which could stimulate growth of granulocyte cells (neutrophil, eosinophil and basophil) and macrophage. The results of this study showed that there is a significant competition between interleukin 3, 5 and GM-CSF in attaching to eosinophil receptors.

REFERENCES


Shahin Gavanji graduated in Biotechnology at MSc at the Department of Biotechnology, Faculty of Advanced Sciences and Technologies, University of Isfahan, Isfahan, Iran. He has over 10 international medals in invention. Shahin Gavanji's research has focused on Pharmacy and Pharmacology, Nano Biotechnology, Bioinformatics, Biotechnology - Medical Biotechnology. He is editor in chief of International Journal of Scientific Research in Inventions and New Ideas.

Dr. Hassan Mohabatkar is a faculty member at Department of Biotechnology, Faculty of Advanced Sciences and Technologies, University of Isfahan, Isfahan, Iran. His research has focused on Bioinformatics.

h.mohabatkar@yahoo.com