Homology Modelling of Chemerin like Receptor-1 (CMKLR1): Potential Target for Treating Type II Diabetes

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Abstract

Chemerin receptor, which predominantly expressed in immune cells as well as adipose tissue, was found to stimulate chemotaxis of dendritic cells and macrophages to the site of inflammation. Chemerin is a widely distributed multifunctional secreted protein implicated in immune cell migration, adipogenesis, osteoblastogenesis, angiogenesis, myogenesis, and glucose homeostasis. Recent studies suggest chemerin may play an important role in the pathogenesis of obesity and insulin resistance and it becomes a potential therapeutic target for treating type II diabetes. The crystal structure of chemerin receptor has not yet been resolved. Therefore, in the present study, homology modelling of CMKLR1 was done utilizing the crystal structure of human angiotension receptor in complex with inverse agonist olmesartan as the template. Since the template has low sequence identity, we have incorporated both threading and comparative modelling approach to generate the three dimensional structure. 3D models were generated and validated. The reported models can be used to characterize the critical amino acid residues in the binding site of CMKLR1.

Keywords: Chemerin, CMKLR1, Homology Modelling, Threading

1. Introduction

Chemerin is a chemoattractant protein that acts as a ligand for the G protein-coupled receptor CMKLR1 (also known as ChemR23 or DEZ) has a role in adaptive and innate immunity[1-2]. Chemerin is a 14 kDa protein secreted in an inactive form as prochemerin and is activated through cleavage of the C-terminus by inflammatory and coagulation serine proteases[3-4]. Chemerin, also known as retinoic acid receptor responder protein 2 (RARRES2), tazarotene-induced gene 2 protein (TIG2), or RAR-responsive protein TIG2 is encoded by the RARRES2 gene in humans[5]. ChemR23 exhibits homology to neuropeptide and chemoattractant receptors and is expressed in monocyte-derived dendritic cells, macrophages, antigen presenting cells and, at a lower expression level, in CD4 T lymphocytes[6-8]. Chemerin was found to stimulate chemotaxis of dendritic cells and macrophages to the site of inflammation[9]. The estimated concentration of active chemerin in plasma and serum, respectively, was 3.0 and 4.4 nM in humans. In humans, chemerin mRNA is highly expressed in white adipose tissue, liver and lung while its receptor, CMKLR1 is predominantly expressed in immune cells as well as adipose tissue[10]. Because of its role in adipocyte differentiation and glucose uptake, chemerin is classified as an adipokine. Interaction of chemerin like receptor-1 with RARRES2 induces activation of intracellular signaling molecules, such as SKY, MAPK1/3 (ERK1/2), MAPK14/P38MAPK and PI3K leading to multifunctional effects, like, reduction of immune responses, enhancing of adipogenesis and angiogenesis. Studies in mice have shown neither chemerin nor CMKLR1 are highly expressed in brown adipose tissue, indicating that chemerin plays a role in energy storage rather than thermogenesis. A recent finding on macrophages have shown that it has been implicated in chronic inflammation of adipose tissue in obesity which implies chemerin may play an important role in the pathogenesis of obesity and insulin resistance[11,12]. Chemerin is a widely distributed multifunctional secreted protein implicated in immune cell migration, adipogenesis, osteoblastogenesis, angiogenesis, myogenesis, and glucose homeostasis[13,14]. Also, Chemerin plays a role in insulin sensitivity and may be...
a potential therapeutic target for treating type II diabetes.

The three dimensional structure of CMKLR1 is an essential component in understanding its underlying biological functions at molecular level. In the current study, comparative modelling and threading based model generation was done for CMKLR1 receptor because the three-dimensional structure of chemerin receptor is not yet available. Fourteen models were successfully generated, and validated using ERRAT and Ramachandran plot scores. The validation results obtained were acceptable and used to identify and suggest the best model for performing the further docking studies for the identification of the important binding site residues.

2. Material and Methods

2.1. Template Selection

The amino acid sequence of human chemerin receptor (accession No: Q99788) was retrieved from the Uniprot database. Protein BLAST\cite{15} search was performed against PDB\cite{16} with the default parameters to find suitable templates for homology modelling. The crystal structure of human angiotension receptor in complex with inverse agonist olmesartan was selected as the template. Templates were selected based on sequence identity, query coverage and E-value. Multiple sequence alignment was done using CLUSTALW\cite{17} program to find conserved residues.

2.2. Homology and Threading based Modeling of CMKLR1

The three dimensional structures of CMKLR1 were modeled using EasyModeller 4.0\cite{18} which uses MODELLER 9.12\cite{19} and Python 2.7.1 in the backend. I-Tasser\cite{20} server was also used to perform the modelling of CMKLR1 receptor. I-Tasser is a protein structure modeling approach based on the secondary-
structure enhanced profile-profile threading alignment (PPA) and the iterative implementation of the Threading ASSEmbly Refinement (TASSER) program. In this approach, the target sequence is first threaded through a PDB structure library to search for the possible folds by four simple variants of PPA methods employing the hidden Markov model, PSI-BLAST profiles, Needleman-Wunsch and Smith-Waterman alignment algorithms.

2.3. Validation of CMKLR1

The predicted models were validated using Ramachandran\(^1\)\(^{[21]}\) and ERRAT plot\(^2\)\(^{[22]}\). The Ramachandran plot gives us information about the percentage of residues in allowed region. The ERRAT program is well suited for evaluating the progress of crystallographic model building and refinement and analyzes the statistics of non-bonded interactions between different atom types.

3. Results and Discussion

3.1. Template Selection

In order to identify the adequate template for the target protein CMKLR1, BLAST search was performed against the protein data bank (PDB). The blast search resulted in 43 templates among which the templates identity ranges between 22%-34% with varying query coverage. The crystal structure of human angiotension receptor in complex with inverse agonist olmesartan (4ZUD) was selected as template because it satisfies the template selection criteria’s such as sequence identity >30%, maximum query coverage. The alignment between CMKLR1 and template 4ZUD is shown in Fig. 1.

3.2. Model generation and Validation

The three dimensional structure of CMKLR1 protein was modeled using I-tasser and Easy-Modeller. Five models were obtained from I-Tasser server utilizing ten different templates. Nine models were generated from EasyModeller using 4ZUD as template. Although the identity was small the generated models retain the seven transmembrane regions which is the predicted topology of GPCR family. The validation for 14 models was performed using RAMPAGE tool to calculate the stereochemical properties of the model. The model generated using I-Tasser has more residue in the outlier region (4.6% to 12.5%) than the models generated using Easy-modeller (0.5% to 1.9%). The I-Tasser model has higher quality score in ERRAT plot compared to the models generated using Easy-modeller. ERRAT program was used to analyze the overall quality factor of all the generated models. The quality of the models was in the range of 79%-92% for I-Tasser models and 72% to 83% for the models generated from EasyModeller. The results obtained from Ramachandran plot and ERRAT

| Model No | Software Used | Ramachandran plot | ERRAT quality score |
|----------|---------------|-------------------|---------------------|
| 1        | I-Tasser      | 76.0% 11.3% 12.7% | 79.45               |
| 2        | 84.4% 8.6% 7.0% | 92.87           |
| 3        | 80.6% 12.7% 6.7% | 79.16           |
| 4        | 81.9% 12.7% 5.4% | 88.43           |
| 5        | **81.1%** 14.3% 4.6% | **92.87**     |
| 6        | 93.3% 4.9% 1.9% | 75.34           |
| 7        | 95.1% 3.8% 1.1% | 72.76           |
| 8        | **94.6%** 4.1% 1.3% | **83.28**     |
| 9        | 94.3% 5.1% 0.5% | 79.45           |
| 10       | Easy-Modeller | 93.8% 5.4% 0.8% | 74.52               |
| 11       | 94.9% 4.0% 1.1% | 75.89           |
| 12       | 94.3% 4.6% 1.1% | 79.17           |
| 13       | 96.0% 2.4% 1.6% | 75.89           |
| 14       | 95.1% 4.3% 0.5% | 75.89           |

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plot for the generated models were tabulated in Table 1. Based on the validation result, two models were selected one from comparative modeling (model no 8) and one from threading (model no 5) approach. The model obtained from I-Tasser shows 95.4% of residues in favored and allowed region and has overall quality factor as 92. The best model selected from Easy modeller shows 98.7% of residues in favored and allowed

Fig. 2. 3D structure of the selected best model for human CMKLR1 protein selected from I-Tasser (a) and EasyModeller (b).

Fig. 3. Ramachandran plot for the selected model obtained from I-Tasser (a) and EasyModeller (b).
region and its quality value in ERRAT plot was found to be 83. Fig. 2 represents the three dimensional structure of the selected models. The validation result obtained from Rampage and ERRAT server for the selected models is shown in Fig. 3 and 4 respectively.

4. Conclusion

In this study, the three dimensional model of human chemerin receptor (CMKLR1) was developed using two different modeling approaches namely, homology modeling and threading. Reliable models were constructed using EasyModeller and I-Tasser server and validated using Ramachandran plot and ERRAT plot to explore its biological functions. We hope that our model could serve as cornerstone for identifying the critical residues and for designing new inhibitors against chemerin receptors.
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