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Structural characterization of ANGPTL8 (betatrophin) with its interacting partner lipoprotein lipase

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ABSTRACT

Angiopoietin-like protein 8 (ANGPTL8) (also known as betatrophin) is a newly identified secretory protein with a potential role in autophagy, lipid metabolism and pancreatic beta-cell proliferation. Its structural characterization is required to enhance our current understanding of its mechanism of action which could help in identifying its receptor and/or other binding partners. Based on the physiological significance and necessity of exploring structural features of ANGPTL8, the present study is conducted with a specific aim to model the structure of ANGPTL8 and study its possible interactions with Lipoprotein Lipase (LPL). To the best of our knowledge, this is the first attempt to predict 3-dimensional (3D) structure of ANGPTL8. Three different approaches were used for modeling of ANGPTL8 including homology modeling, de-novo structure prediction and their amalgam which is then proceeded by structure verification using ERRATT, PROSA, Qmean and Ramachandran plot scores. The selected models of ANGPTL8 were further evaluated for protein–protein interaction (PPI) analysis with LPL using CPORT and HADDOCK server. Our results have shown that the crystal structure of iSH2 domain of Phosphatidylinositol 3-kinase (PI3K) p85β subunit (PDB entry: 3mtt) is a good candidate for homology modeling of ANGPTL8. Analysis of inter-molecular interactions between the structure of ANGPTL8 and LPL revealed existence of several non-covalent interactions. The residues of LPL involved in these interactions belong from its lid region, thrombospondin (TSP) region and heparin binding site which is suggestive of a possible role of ANGPTL8 in regulating the proteolysis, motility and localization of LPL. Besides, the conserved residues of SE1 region of ANGPTL8 formed interactions with the residues around the hinge region of LPL. Overall, our results support a model of inhibition of LPL by ANGPTL8 through the steric block of its catalytic site which will be further explored using wet lab studies in future.

1. Introduction

Angiopoietin-like protein 8 (ANGPTL8) is a newly identified secretory protein and is also known as Lipasin (Zhang, 2012), RIFL (Ren et al., 2012), C19orf80 (Lin and Lin, 2014) and Betatrophin (Yi et al., 2013) due to its functional characterization in various studies. Specifically, its role in pancreatic beta-cell proliferation is still controversial and requires further investigation regarding its specific binding partners (Yi et al., 2013; Gusarova et al., 2014; Chen et al., 2015). The physiological regulation of ANGPTL8 is not fully comprehended yet and its precise mechanism of action along with the identity of its receptor is still a quest. To the best of our knowledge, the protein structure of ANGPTL8 and none of the other family members is predicted yet. Therefore, further studies which could elucidate the structural aspects of ANGPTL8 are required in order to identify its receptor and/or other binding partners to help increase our understanding of its mechanism of action and accelerate future studies for translation of this important discovery into clinical practice.

The human gene encoding ANGPTL8 is entitled C19orf80. Its physiological expression is nutritionally regulated; it is induced on refeeding and reduced upon fasting (Ren et al., 2012). It is expressed in both adipose tissue and liver of mice whereas its human expression is liver specific (Zhang, 2012). The thyroid hormone 3,3′,5-triido-L-thyronine (T3) upregulates the expression of ANGPTL8 in human liver cells (Lin and Lin, 2014). This induction occurs through thyroid hormone recptors alpha and beta (THR-α and THR-β) which make heterodimers with Retinoid-X Receptor

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(RXR), to transactivate ANGPTL8. The gene regulation of ANGPTL8 is comparable with similar family members such as ANGPTL3 and ANGPTL4 which are transactivated by the formation of heterodimers of different nuclear receptors (LXR-RXR for ANGPTL3 and PPAR-RXR for ANGPTL4) (Kersten, 2005). The expression of ANGPTL8 in adipocytes is upregulated by insulin and downregulated by cAMP (Ren et al., 2012). ANGPTL8 has been characterized as an atypical member of ANGPTL protein family because of the presence of only an N-terminal (coiled-coil) domain (Fu et al., 2013; Hato et al., 2008; Santulli, in press). The seven typical ANGPTL protein family members containing an N-terminal (coiled-coil) domain and a C-terminal (fibronogen-like) domain with different roles associated with each domain are comprehensively discussed in (Fu et al., 2013; Hato et al., 2008; Santulli, in press). The phylogenetic analysis of ANGPTL8 revealed that ANGPTL3 and ANGPTL4 are its closest family members while sequence alignments expressed significant similarity of ANGPTL8 with the N-terminal domain (region known for binding and inhibition of Lipoprotein lipase (LPL)) of these proteins (Zhang, 2012; Ono et al., 2003; Koster et al., 2005).

Several studies have demonstrated the role of ANGPTL8 in the lipid metabolism by regulating the level of plasma triglycerides (TG) and cholesterol through inhibition of LPL (Zhang, 2012; Ren et al., 2012; Wang et al., 2013). LPL is an enzyme which regulates lipid homeostasis by breaking down the dietary lipids for subsequent uptake in peripheral tissues. The physiological tissue specific distribution and activity of both LPL and ANGPTL8 are differentially regulated under different nutritional states to maintain plasma TG levels (Zhang, 2012; Ren et al., 2012; Kersten, 2014). Both gain- and loss-of-function studies consistently demonstrated the role of inhibition of ANGPTL8 as a therapeutic strategy for dyslipidemia by reducing the plasma TG levels in correlation with high LPL activity (Zhang, 2012; Ren et al., 2012; Wang et al., 2013; Fenzl et al., 2014).

(Yi et al., 2013) supported the role of ANGPTL8 as a potent stimulator of pancreatic beta-cells in insulin resistant mouse model and thus, regulating glucose metabolism. Their data provided substantial evidence that pancreatic beta-cell proliferation and improved glucose tolerance in response to ANGPTL8 induction is itself independent of insulin resistance and occurs as a compensatory mechanism. The ANGPTL8 mediated adult and aged pancreatic beta-cell proliferation in a rat model through targeted delivery of ANGPTL8 gene further augments this role, despite of the ongoing controversy on the role of ANGPTL8 in proliferation of pancreatic beta-cells (Chen et al., 2015; Gusarova et al., 2014). Therefore, the potential of ANGPTL8 in regenerative therapeutics of Diabetes Mellitus aiming the restoration of pancreatic beta-cell mass still exists.

In the light of above-mentioned studies, ANGPTL8 is emerging as a potential drug target for both Dyslipidemia and Diabetes Mellitus which necessitates the prediction of its structural aspects in order to enhance current understanding of its mode of action and identify its activity partners. The structural characterization of ANGPTL8 is thus a pre-requisite which may lead to subsequent studies in drug designing and development. Based on the physiological significance and necessity of exploring structural features of ANGPTL8, the present study is conducted with a specific aim to construct the structure of ANGPTL8 and to study the possible interactions of ANGPTL8 with LPL. To the best of our knowledge, this is the first attempt to generate a 3-dimensional (3D) structure of ANGPTL8. We have used computational strategy which include sequence analysis, secondary structure prediction, comparative modeling and its validation through protein–protein interactions (PPI) analysis. The study provides few of the very first instances to understand and to elucidate some of the functional roles associated with ANGPTL8. The predicted model is in coherence with previous studies.

2. Methodology

2.1. Sequence alignment analysis of ANGPTL8

Sequences of ANGPTL8 (Uniprot entries: Q6UXH0 and Q8R1L8 for human and mouse respectively), N-terminal domain of ANGPTl3 (Uniprot entries: Q9Y5C1 and Q9R182 for human and mouse respectively) and N-terminal domain of ANGPTL4 (Uniprot entries: Q9BY76 and Q9Z1P8 for human and mouse respectively) were aligned using multiple sequence alignment server ClustalW2 (McWilliam et al., 2013). Known binding and regulatory regions of ANGPTL3 and ANGPTL4 were investigated for their presence in ANGPTL8.

2.2. Secondary structure prediction of ANGPTL8

Secondary structure prediction of protein refers to a set of techniques which aim to predict the location and number of alpha helices and beta strands within a protein or protein family. It has long been recognized that patterns of residue conservation are indicative of particular secondary structure types. We used several secondary structure prediction methods to predict the secondary structure of ANGPTL8 which includes PSIPRED (McGuffin et al., 2000), Predict Protein Server (Rost et al., 2004), Jpred server (Cuff et al., 1998), ASPSSP2 (Advanced Protein Secondary Structure Prediction Tool) (Raghava, 2002), and Proteus2 (Montgomerie et al., 2008).

2.3. Comparative structure modeling of ANGPTL8

The complete methodological sequence of events followed in comparative structural modeling of ANGPTL8 is depicted in the flowchart below (Fig. 1). The details of each structure prediction method is given below in subsections.

Standard procedure for comparative modeling of ANGPTL8 using PSI-BLAST searches was proceeded by several other protein structure prediction servers including I-TASSER (Yang et al., 2015), PHYRE^2 (Kelley and Sternberg, 2009), MUSTER (Wu and Zhang, 2008), QUARK (Xu and Zhang, 2012) and Robetta (Kim et al., 2004) due to the lack of optimal structure verification scores for generated models. The details of models generated from I-TASSER, PHYRE^2, MUSTER and QUARK servers are not included here due to insignificant results and space constraints.

2.3.1. APPROACH 1 (A1) for prediction of ANGPTL8

The human protein sequence of ANGPTL8 was retrieved from UniProt with accession number “Q6UXHO”. Next, sequence similarity search was performed using PSI-BLAST (Position-Specific Iterated BLAST) (Altschul and Koonin, 1998) against Protein Data Bank (PDB) (Berman et al., 2000) to retrieve similar protein sequences with known three-dimensional structures to serve as a template for homology modeling. Sequence alignment between the sequences of selected BLAST hits and ANGPTL8 were performed using multiple alignment tool Expresso (Armougom et al., 2006). These alignments were then used to predict the 3D structure of ANGPTL8 using MODELLER (Webb and Sali, 2014). One hundred models were generated by using MODELLER which uses variable target function method (VTFM) in Cartesian space with conjugate gradients (CG) along with molecular dynamics (MD) with simulated annealing (SA) (ali, 1995). The final model selection was based on structure assessment scores calculated by MODELLER, Qmean (Benkert et al., 2009) PROSA (Wiederstein and Sippl, 2007), ERRAT (Colovos and Yeates, 1993), and RAMPAGE server (Lovell et al., 2003)). ERRAT score provides a confidence score in terms of non-bonded atom-atom interactions in comparison with already reported highly reliable structures (Colovos and