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Prediction of post translation modifications at the contact site between *Anaplasma phagocytophilum* and human host during autophagosome induction using a bioinformatic approach

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ABSTRACT

Autophagy is crucial for maintaining physiological homeostasis, but its role in infectious diseases is not yet adequately understood. The binding of *Anaplasma* translocated substrate-1 (ATS1) to the human Beclin1 (BECN1) protein is responsible for the modulation of autophagy pathway. ATS1-BECN1 is a novel type of interaction that facilitates *Anaplasma phagocytophilum* proliferation, leading to intracellular infection via autophagosome induction and segregation from the lysosome. Currently, there is no report of post translational modifications (PTMs) of BECN1 or cross-talk required for ATS-BECN1 complex formation. Prediction/modeling of the cross-talk between phosphorylation and other PTMs (*O*-β-glycosylation, sumoylation, methylation and palmitoylation) has been attempted in this study, which might be responsible for regulating function after the interaction of ATS1 with BECN1. PTMs were predicted computationally and mapped onto the interface of the docked ATS1-BECN1 complex. Results show that BECN1 phosphorylation at five residues (Thr91, Ser93, Ser96, Thr141 and Ser234), the interplay with *O*-β-glycosylation at three sites (Thr91, Ser93 and Ser96) with ATS1 may be crucial for attachment and, hence, infection. No other PTM site at the BECN1 interface was predicted to associate with ATS1. These findings may have significant clinical implications for understanding the etiology of *Anaplasma* infection and for therapeutic studies.

1. Introduction

Anaplasma phagocytophilum is a facultative, intracellular, rickettsial pathogen which infects domestic ruminants and a wide range of mammalian hosts [1,2]. Among them, sheep, cattle and goats are common definitive hosts, while the common zoonotic vector is a tick of the genus *Ixodes* [2]. Varying symptoms are observed in different infected hosts, and it causes granulocytic anaplasmosis in humans through infection of white blood cells and increasing their life span [3]. A type IV secretion system (T4SS) apparatus facilitates transfer of molecules between the bacterium and the host [4]. The bacterium survives by obtaining nutrients in the endosome and by inducing autophagy [5]. Rapamycin induction of autophagy has established that inclusion-targeted autophagosome development supports *A. phagocytophilum* replication [6]. Autophagy induction occurs by bacterial ATS1 binding to the human BECN1, after which the BECN1-Atg14L autophagy initiation pathway is redirected to acquire host nutrients for its own growth [7]. Despite expression of all of the *A. phagocytophilum* T4SS proteins in the leukocytes during anaplasmosis, only T4SS effectors, an ankyrin repeat-rich protein A (AnkA) and ATS1 alter host autophagy mechanism in favour of the bacterial growth [7–10]. On the whole, *A. phagocytophilum* hijacks the host system, suppresses the innate immune response and boosts cholesterol uptake to aid survival [9].

A dual role for ATS1 has been proposed [7]. One portion of ATS1 with a mitochondria-targeting presequence translocates across the outer and inner membranes. The presequence is shed, and the

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mature ATSl localizes in the mitochondrial matrix. Mitochondria-localized ATS1 benefits autophagy by delayed apoptosis of host cells. This is accomplished by inhibiting loss of mitochondrial membrane potential. Other portion of ATS1 interacts with the host autophagosome initiation complex (Atg14L-BECN1-Vps34) and stimulates omegasomes in the endoplasmic reticulum. ATS1 is a unique example of a bacterial BECN1 binding protein that takes over the BECN1-Atg14L autophagy initiation pathway, likely to provide nutrients for bacterial growth [7].

The role of phosphorylation is in the regulation of autophagy by ABl1 and, consequently, AnkA has been proposed, but information is lacking for this type of modification on BECN1 as well as its impact on ATS1 during autophagy. Phosphorylation has been proposed at the human ABl1 protein interface via ABl1 tyrosine kinase, which in turn phosphorylates the T4SS effector AnkA at two sites. Other AnkA sites are phosphorylated by Src kinases, which lead to binding of AnkA to the SH2 domain of Shp-1 protein. AnkA protein is then translocated to the nucleus and regulates genes [10].

Phosphoryation is the most important post-translational modification, i.e., the covalent addition of a phosphate group to an amino acid, and impacts functional regulation of proteins [11]. Here, we studied the phosphorylation, other possible PTMs and their cross-talk on BECN1 that might impact on attachment to ATS1 and proliferation. It is difficult and costly to assess this type of cross-talk experimentally, so that an *in silico* approach was employed. A study of phosphorylation and *O*-β-gycosylation interplay using computational tools has previously provided insight into human cellular processes and biological activity as transcription [12], gene expression and virulence potential of human pathogens with implications for therapies in the case of diseases [13]. Artificial neural network (NN) and support vector machine (SVM) based methods are available for predicting various PTMs with substantial precision [14]. This study provides novel insights into the mechanism of bacterial induced autophagy and its modulation of host machinery to cause infection/disease.

2. Material and methods

Sequences for human BECN1 (Accession ID: Q14457) and *A. phagocytophilum* ATS1 (Accession ID: Q2GJL5) were retrieved from the Uniprot database.

2.1. Sequence analysis

Protein sequences were subjected to an array of analytical methods utilizing various software programs and webservers, in order to understand detailed features, evolution, structure and function during ATS1-BECN1 contact. To assess surface accessibility of BECN1 residues necessary for interaction with ATS1, NetSurfP v1.1 [15] was used. The accuracy of each calculation was determined in the form of a Z-score based on neural networks, which makes efficacy of the prediction reliable. Multiple sequence alignment was carried out using ClustalO (http://www.ebi.ac.uk/Tools/msa/clustalo/) to study sequence conservation analysis in two model species. The BECN1 sequence of *Homo sapiens* (human) was compared to those of *Mus musculus* (mouse) and *Rattus novergicus* (rat) BECN1, with Uniprot accession numbers: O88597 and Q91XJ1, respectively. Secondary structure analyses of BECN1 and ATS1 were conducted using the Polyview server [16]. The conserved domain database (http://www.ncbi.nlm.nih.gov/Structure/cdd) of NCBI was explored for studying protein domains.

2.2. PTM assessment

Phosphorylation was studied using NetPhos 2.0 [17,18]. This server estimates phosphorylation sites for serine, threonine and tyrosine residues in eukaryotic proteins. Kinases for these phosphorylated sites were determined employing NetPhosK 1.0 [18] and Kinase Phos 2.0 [19] servers that utilize NN and SVM approach. NetPhosK 1.0 includes seventeen kinases (PKA, PKC, PKG, CKII, Cdc2, CaM-II, ATM, DNA PK, Cdk5, p38 MAPK, GSK3, CKI, PKB, RSK, INSR, EGFR and Src), whereas Kinase Phos 2.0 covers 71 kinases. Phosphosites were also mined from the PhosphoELM database [20]. Acetylation was assessed using the NetAcet 1.0 server [21], which reveals substrate residues of N-acetyltransferase A. The prediction algorithm is centered on experimental yeast dataset values, but is known to perform well also for mammalian substrate residues that might be acetylated by NatA orthologs. Methylation modification due to the action of methyl transferases was predicted on arginine and lysine residues by MEMO (http://www.bioinfo.tsinghua.edu.cn/~tigerchen/memo.html). Palmitoylation was predicted by CSS-Palm 4.0 (http://csspalm.biocuckoo.org/online.php). *O*-GlcNAcylation was studied using NetOGlyc webserver [22]. The interplay of phosphorylation and *O*-β-GlcNAcylation was predicted using the YinOYang server [23]. Ubiquitination was predicted using BDM-PUB (http://bdmpub.biocuckoo.org/prediction.php), and GPS-SUMO [24] was used for predicting sumoylation at lysine residues.

2.3. Structure prediction and molecular docking study

The structure of human BECN1 is available in the Protein Data Bank (www.rcsb.org). However, to model the protein folds for the region (amino acid residues 1–272) responsible for the interaction with *A. phagocytophilum* ATS1 [7], the complete structure of the BECN1 protein (450 amino acids) was predicted using I-TASSER [25,26]. This server detects templates from the Protein Data Bank by multiple threading method called LOMETS [27], and uses repeated assembly and simulation of template fragments for full-length protein model prediction. Among the predicted models, the best one with the highest C-score and a low root mean square deviation was selected for further analysis, as described previously [28].

The structure of ATS1 has not yet been solved, and has very low homology to existing X-ray and NMR structures in the Protein databank; therefore, it was predicted employing an *ab initio* approach via CABS-fold server (http://biocomp.chem.uw.edu.pl/CABSfold/index.php) [29,30]. In addition to root mean square deviation, CABS-fold provides global distance test value for the selection of the best, predicted protein structure (higher values for better structures). These criteria were used for the selection of structures for analysis.

The molecular docking of the BECN1-ATS1 complex was performed using High Ambiguity Driven biomolecular DOCKing (HADDOCK), which takes into account biochemical, biophysical or amalgamated data of both properties [31]. Important residues i-e mid-section of ATS1 (amino acids 90–250) and one-half of human BECN1, including N-terminal (amino acids 1–272) for interaction (determined previously *in vivo* by co-immunoprecipitation, confirmed by reciprocal co-immunoprecipitation [7]), were fed into the HADDOCK server as active site residues. Passive residues were automatically defined around the active site-interacting residues. Other parameters were kept as default.

3. Results and discussion

Autophagy, a central macromolecule and organelle degradation process for the maintenance of cell and tissue homeostasis, is vital for cell regulation and allied to numerous human diseases [32]. BECN1 (along with the mammalian class III phosphatidylinositol 3-kinase and Vps34) is an imperative constituent of a complex