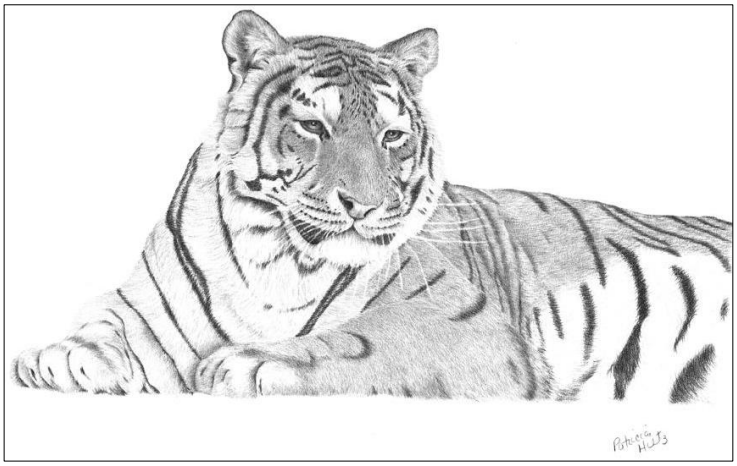
Bioinformatics Study on a Pathology-Associated Sequence

Abstract

The incidence of symptoms indicative of vitamin A deficiency in a population of Siberian tigers, despite sufficient vitamin intake, prompted an investigation into potential genetic causes. A bioinformatics approach was adopted to analyze an overexpressed 783 bp mRNA sequence from liver biopsies, identified as part of the RBP4 gene, which is crucial for retinol transport. Comparative sequence analysis via BLAST and multiple alignments using CLUSTAL OMEGA revealed several mutations, with the A46I* mutation of particular interest due to its location in a region that potentially affects retinol binding. Subsequent homology modeling using Swiss Model, followed by structural alignment in PyMOL against a known RBP structure (PDB ID: 1RBP), illustrated a conserved overall fold but suggested an altered retinol-binding pocket in the mutated protein. While the structural models implicate the A46I* mutation in the malfunction of retinol transport, a lack of experimental validation necessitates caution in the interpretation of these results. The findings, therefore, provide a direction for future research, which should include functional assays to verify the impact of the identified mutations on RBP4's activity and the health of Siberian tigers.

Keywords: Siberian Tigers, RBP4, Vitamin A Deficiency, Bioinformatics, Homology Modeling, Mutation Analysis, Protein-Ligand Binding.



Introduction

In the context of a study on a cohort of Siberian tigers exhibiting symptoms indicative of vitamin A deficiency—despite adequate nutritional intake—our laboratory was engaged to explore the molecular underpinnings of this anomaly. Preliminary investigations uncovered the overexpression of an mRNA sequence, spanning 783 base pairs, in the liver tissues of the affected individuals, suggesting a potential malfunction in vitamin A metabolism. The focal point of our research is Retinol-Binding Protein 4 (RBP4), a pivotal transporter of vitamin A in the bloodstream, integral for vision and the regulation of gene expression. Under normal circumstances, RBP4 mediates the delivery of retinol to various tissues. However, the observed overexpression in our subjects may indicate a transport or metabolic dysfunction of vitamin A, contributing to the pathological symptoms. This revelation paves the way for more in-depth analyses to elucidate the connection between this overexpression and the health of the tigers, with the aim of identifying potential interventions to ameliorate their condition.

Materials and Methods

BLAST Analysis

To identify the molecular underpinnings of the pathology observed in Siberian tigers, we commenced our investigation with a BLAST (Basic Local Alignment Search Tool) search. This initial step aimed to find the closest sequence match to our mRNA of interest, a 783 base pair segment significantly overexpressed in the liver tissues of the affected tigers. Utilizing the non-redundant (NR) protein sequences database, which comprises hundreds of millions of sequences, allowed us to cast a wide net in our search for related sequences. For more refined results, we also employed the SWISSPROT database, known for its well-annotated sequence entries, facilitating a focused investigation into proteins directly associated with the observed pathology. This approach helped in hypothesizing how certain amino acid mutations—either silent (MUT SIL) or pathogenic (MUT PATH) —could contribute to the disease mechanism.

Selection of Sequences for Multiple Alignment

After the BLAST analysis identified closely related sequences, we proceeded to select a diverse array of sequences from various mammals for a multiple sequence alignment, aiming for 10 sequences in total. This selection aimed to cover a broad phylogenetic spectrum, including sequences from different types of mammals such as fish, cats, and humans, to provide a comparative perspective on the evolutionary conservation of the sequence of interest. After the initial BLAST analysis identified the overexpressed mRNA's closest matches, we selected sequences from a broad range of mammals for multiple sequence alignment. This selection included:

- Domesticated animals (Bos taurus, Felis catus),
- Wild carnivores and omnivores (Hyaena hyaena, Gorilla gorilla gorilla, Mustela erminea, Ursus maritimus),
- Specialized feeders (Phyllostomus discolor),
- Aquatic and semi-aquatic mammals (Hippopotamus amphibius kiboko, Callorhinus ursinus),
- Humans (Homo sapiens).

This diverse selection was aimed at encompassing a wide evolutionary spectrum to understand the conservation of the sequence across different lineages. The alignment, conducted via CLUSTAL OMEGA, aimed to pinpoint mutations unique to the pathological sequence, shedding light on potential disease mechanisms at the molecular level.

Structural Analysis

With potential pathogenic mutations identified, our next step was to assess their impact on the protein's structure and function. To predict the tertiary structure of the protein encoded by the overexpressed mRNA, we employed SWISSMODEL, an automated homology-modeling server. This enabled us to infer the structural deviations that might arise from the identified mutations. Furthermore, using PyMOL for structure alignment, we examined the precise localization of these mutations—whether they occurred on the N-terminus, within coil regions, or at binding sites.

Results and Discussion

Identification of the RBP4 Sequence

Using the translated mRNA sequence provided for BLAST analysis, we identified the sequence as corresponding to Retinol-Binding Protein 4 (RBP4) from Siberian tigers. This protein is known to be a critical transporter of vitamin A, vital for numerous biological functions including vision. The significant overexpression of RBP4 in the affected tigers suggests a potential malfunction in vitamin A transport and metabolism, which could underlie the observed symptoms of deficiency despite adequate dietary intake.

Pathological Annotation

An examination of the RBP4 sequence revealed several amino acid substitutions when compared to the reference sequences from other mammals. These mutations are potentially relevant to the observed pathology.

Comparative Sequence Analysis

We conducted a multiple sequence alignment using the retrieved sequences from diverse mammalian taxa, including representatives from both carnivorous and non-carnivorous lineages, to ascertain the conservation of RBP4 and to identify any mutations unique to the pathological state. The alignment, carried out through CLUSTAL OMEGA, indicated the presence of several point mutations exclusive to the Siberian tiger sequence, as detailed in the provided alignment visualization (Figure 1).

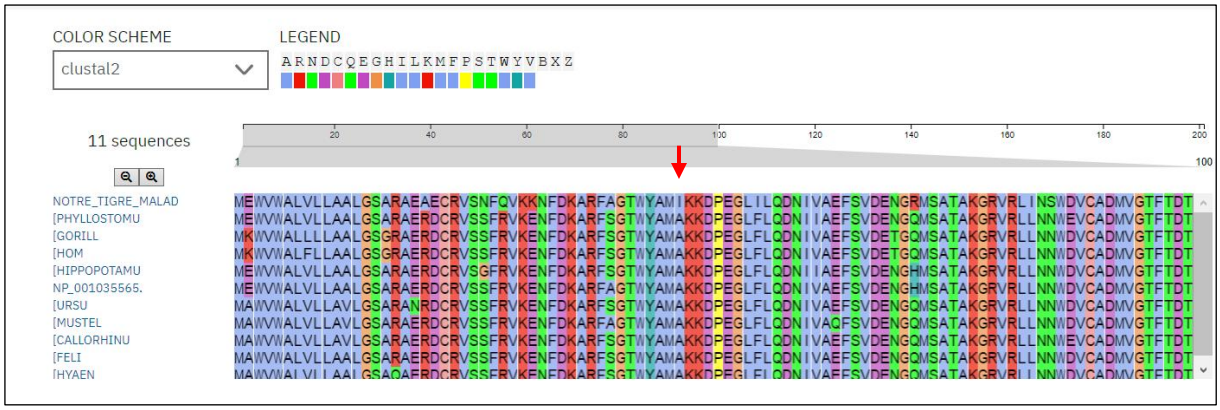


Figure 1: Multiple Sequence Alignment of Retinol-Binding Protein 4 (RBP4) Across Selected Mammalian Species

Mutation Analysis

A detailed investigation into the RBP4 gene of the affected Siberian tigers revealed a series of amino acid substitutions. Table 1 encapsulates these mutations, charting the differences from the wild-type sequence and postulating their possible consequences for the protein's structural integrity and functionality. The mutations identified, with positions and changes listed as follows, indicate varied potential impacts on the protein structure and function:

Position	Wild-Type aa	Mutated aa	Potential Impact
20	R	A	Reduced stability or altered charge interactions
26	N	S	Possible loss of glycosylation site
28	R	Q	Potential loss of positive charge
31	E	K	Charge reversal
46	A	I*	Increased hydrophobicity and size
54	I	F	Altered hydrophobic interactions
70	Q	R	Potential gain of positive charge
84	S	N	Possible introduction of glycosylation site
134	I	V	Slight change in hydrophobicity

Table 1: Summary of RBP4 Sequence Mutations and Their Potential Impacts

Structural Implications of Mutations

To gain insight into the structural implications of the identified mutations in RBP4, we utilized Swiss Model, an automated homology modeling server. Homology modeling is predicated on the principle that three-dimensional (3D) structures of proteins within the same family are more conserved than their amino acid sequences. Given the high sequence identity of 87.29% between our target mRNA sequence from the Siberian tiger and the template structure of retinol-binding protein from pig plasma (PDB: 1aqb.1.A), Swiss Model provided a suitable framework for predicting the 3D structure of the tiger's RBP4 (Figure 2). The tertiary structure model of RBP4 reveals the presence of a prominent alpha-helix and a beta-barrel (beta-sheets arranged in a barrel-like structure), typical features of the retinol-binding protein family.

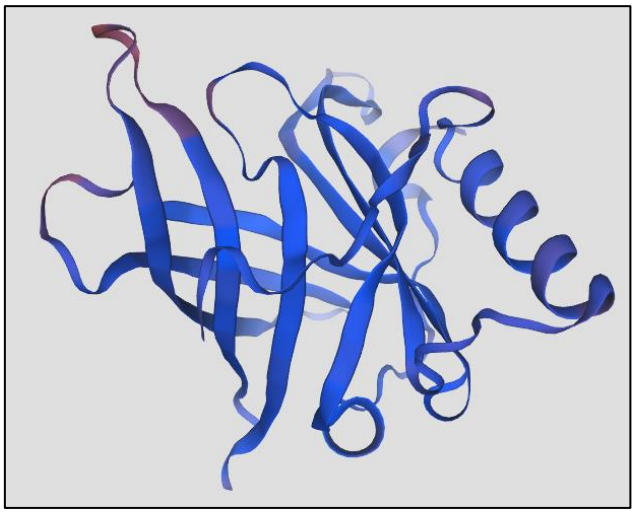


Figure 2: Homology Model of Siberian Tiger RBP4 Predicted by Swiss Model

To further our understanding of the structural implications of the mutations identified in the Siberian tiger RBP4, we employed PyMOL to conduct a comparative analysis. We obtained our predicted RBP4 model (model_02) and the reference structure of retinol-binding protein containing the retinol ligand (PDB ID: 1RBP). Using PyMOL, we have aligned our predicted model of RBP4 from the Siberian tiger (shown in green) with the reference retinol-binding protein structure (shown in cyan) from the PDB (Protein Data Bank) entry 1RBP (Figure 3). The alignment reveals a high degree of structural congruence between the two, indicating that the overall folding

pattern of the tiger's RBP4 remains consistent with the known structure. Notably, the ligand retinol (RTL), depicted in the reference structure, resides within the beta-barrel, which is crucial for its biological function. This comparison is essential to understand whether the mutations we've identified could affect the ligand-binding ability of the protein.

Figure 3: Structural Alignment of Predicted RBP4 with Reference Protein

The comparison reveals a notable disparity in how the ligand is situated within the binding pocket between the two structures (Figure 4). This figure illustrates the positioning of the retinol ligand (shown in orange) within the binding cavities of the wild-type (WT) and mutant (MT) RBP4 protein structures. In the wild-type structure, the ligand is snugly nestled within the beta-barrel, indicative of a proper functional state. Contrastingly, in the mutant structure, the ligand appears improperly accommodated, suggesting potential disruptions in binding affinity and, subsequently, in the protein's retinol transport function.

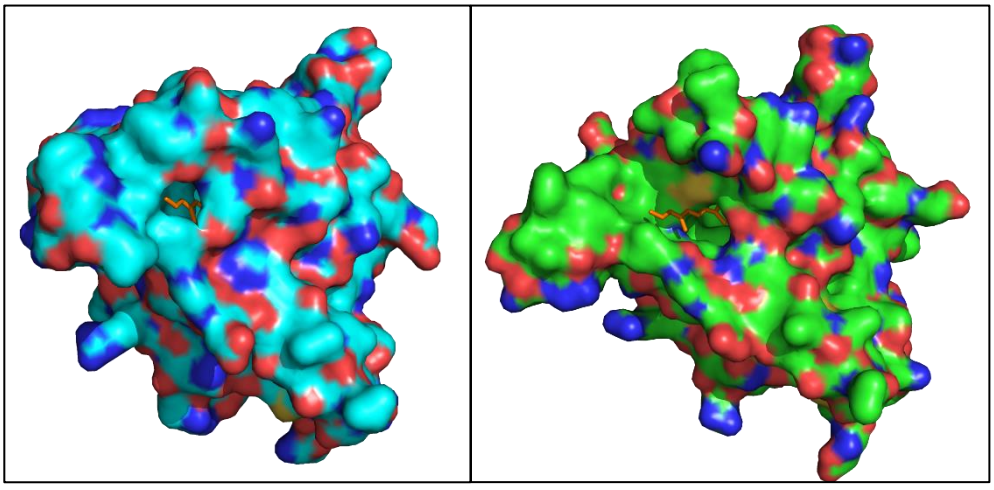


Figure 4: Comparison of Ligand Binding in Wild-Type and Mutant RBP4 Structures, RTL in orange.

Conclusion

Concluding our investigation, we have identified the A46I* mutation in the RBP4 gene as a likely molecular determinant for the vitamin A transport dysfunction observed in Siberian tigers. The structural analyses suggest that the increased hydrophobicity and the change in size at the ligand-binding site are probable contributors to the pathological state. Our findings depict a scenario where the mutated RBP4 may exhibit diminished affinity or altered transport efficiency for retinol, potentially underpinning the clinical manifestations.

However, it is important to acknowledge the limitations inherent in our study. While bioinformatics tools like Swiss Model and PyMOL are powerful for predicting structural consequences of mutations, they are, at their core, predictive models. Our conclusions are drawn from the best available computational and structural data, but they do not substitute for empirical validation. Experiments such as in vitro binding assays, functional complementation tests, and in vivo studies would be necessary to definitively ascertain the role of the A46I* mutation in the pathology of these tigers.

Therefore, although our data strongly implicate the A46I* mutation as a plausible causative factor, we must consider the complexity of biological systems where multiple factors often interplay to result in a phenotypic outcome. Thus, our findings should be viewed as a probable piece of the puzzle, forming a hypothesis that sets the stage for further experimental validation. Our research provides a solid foundation for additional studies, which are crucial to unravel the intricate mechanisms affecting vitamin A metabolism in this endangered species.

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