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Deciphering Protein Drug Targets for Mucormycosis: A Computational Approach

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Abstract
A serious public health issue worldwide, Mucormycosis is a potentially fatal fungal infection that is especially dangerous for people with weakened immune systems. Developing successful treatment plans requires identifying possible protein targets for therapeutic action against the substances causing Mucormycosis. Here, we describe a methodical approach to in silico protein target discovery in Mucorcircinelloides, the causative agent of Mucormycosis. To find candidate proteins necessary for these infections' survival and pathogenicity, we analyzed proteomic data using subtractive proteomics approach aided by bioinformatics tools and databases. To rank possible therapeutic targets, we used sequence analysis, protein-protein interaction prediction, and functional annotation.

Keywords: Mucormycosis, Mucorcircinelloides, Proteomics

Introduction

Like bacteria, fungi play a crucial role in decomposing organic matter, releasing essential elements such as carbon, oxygen, nitrogen, and phosphorus into the soil and atmosphere. They are integral to numerous household and industrial processes, notably in the production of bread, wine, beer, and certain types of cheese. Furthermore, fungi serve as a source of nourishment, with certain species like mushrooms, morels, and truffles esteemed as culinary delicacies. Moreover, mycoproteins derived from the mycelia of specific fungi species are utilized to create protein-rich food products **DePauw et al. (2011).**

*Mucor*spp. are fungi that cause the group of infections known as zygomycosis. Although the term mucormycosis has previously been used to describe this syndrome, zygomycosis is now the preferred term for this angio-invasive disease. Zygomycosis can cause mucocutaneous and rhinocerebral infections, as well as septic arthritis, dialysis-associated peritonitis, renal infections, gastritis, and pulmonary infections. Diabetic ketoacidosis and immunosuppression are the most common risk factors. Desferoxamine treatment, renal failure, extensive burns, and intravenous drug use may all contribute to the development of zygomycosis. The most frustrating aspects of these infections are vascular invasion, which causes necrosis of the infected tissue, as well as perineural invasion. Because of its relatively limited activity, itraconazole prophylaxis in immunosuppressed patients may select the fungi in phylum Zygomycota as the cause **Husain et al (2017)**. Mucormycosis, a life-threatening fungal infection, has emerged as a significant public health concern globally, particularly in immunocompromised

individuals. Identifying potential protein targets for therapeutic intervention against the causative agents of Mucormycosis is crucial for developing effective treatment strategies.

Methodology

Protein Sequence Retrieval

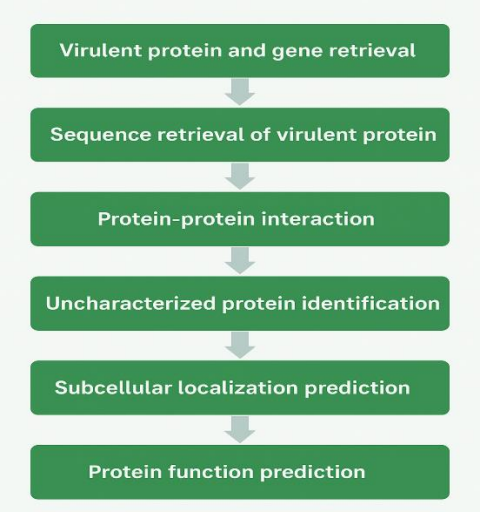
To investigate the proteins involved in Mucor circinelloides, particularly in the context of mucormycosis, a comprehensive list of protein sequences was retrieved from two major publicly available databases: the National Center for Biotechnology Information (NCBI) and the Universal Protein Resource (UniProt). These sequences were downloaded in FASTA format, which is a standard format for representing nucleotide or peptide sequences. UniProt was specifically chosen for its reliability and accuracy in providing a central resource for protein sequences and functional annotation. It integrates data from multiple sources, offering curated information on protein function, domain structure, and sequence features. Similarly, NCBI provides a rich resource for sequence data, including genomic and proteomic datasets, allowing access to annotated proteins of Mucor circinelloides through its protein database.

Functional Annotation and Domain Classification

To classify and functionally annotate the retrieved protein sequences, InterPro was utilized. InterPro is a powerful protein analysis resource that integrates predictive models, known as signatures, from multiple member databases such as Pfam, PRINTS, PROSITE, SMART, TIGRFAMs, and others. These signatures allow for the identification and classification of proteins into families, and prediction of important structural or functional domains and active sites. By running the FASTA sequences through InterProScan, a tool provided by the InterPro database, each protein was analyzed to determine its likely function, domain architecture, and potential involvement in cellular processes.

Data Analysis and Integration

The outputs from InterProScan were cross-referenced with UniProt and NCBI annotations to ensure accuracy and consistency in domain predictions and functional assignments. Proteins were categorized based on domain presence, family classification, and biological relevance to mucormycosis pathogenesis. The analysis focused on identifying proteins with domains commonly associated with virulence, cell wall biosynthesis, immune evasion, and signal transduction pathways. Additionally, the functional classification aided in prioritizing candidate proteins for further structural and docking studies.



Results & Discussion

The primary goal of our research is to identify novel targets for developing potential drugs against Mucorcircinelloides. The subtractive proteomic analysis of the entire mucorcircinelloides was carried out using a variety of database and computational tools, including UniProt, NCBI, STRING database, DeepLoc1.0, and interPro.In our study, we extracted virulent proteins and genes from research papers and literature. A total 18 proteins were included in the network. The protein-protein interaction of virulent proteins was studied using computational tools and databases such as STRING. From its study, we also found some uncharacteristic proteins CHI1S2IYG5, CHI1S2JQZ4, PPS1S2JTA0, GPA1S2JAC5,GPA1S2JRE4,COTHS2JW22,COTHS2JKJ7,COTHS2JJH9,COTHS2ITM4,SSF1S2JNV3,HSS1S2J841,GPR78, S2JPS4.

NO.	PROTEIN	GENE	FACTORS	PATHWAY	MECHANISMS
1.	Ft1	Ft1	Key Virulence	Electron Transport Chain	Rhizopusdelemar
2.	Fet	Fet3a	Virulence	Calcineurin	*M.circinelloides*
3.	Ftr1	Fet3b	Arfs	Rnai	Lichtheimia
4.	Fob1	Fet3c	Zcfs	Sirnas-Esrnas	Corymbifera
5.	Fob2	Cnbr	Tac1	Epimutational	Sophisticated
6.	RNAi	CnaA	Mrr1	Canonical	iron uptake
7.	R3B2,	PkaR	Mdr1	Non-Canonical	cAMP enzyme
8.	Y129F	pkaR1	ADP-Ribosylation	Signaling	Epigenetic
9.	Y140F	pkaR4	Tfs	Pro-Apoptotic	RNAi
10.	CYP51 F5	pkaR2	Lcor02851.1	Apoptotic	Putative

Table -Details account of virulent protein

STRING is a database of known and predicted protein-protein interactions. The interactions include direct (physical) and indirect (functional) associations; they stem from computational prediction, from knowledge transfer between organisms, and from interactions aggregated from other (primary) databases. In STRING, each protein-protein interaction is annotated with one or more 'scores'. Importantly, these scores do not indicate the strength or the specificity of the interaction. Instead, they are indicators of confidence, i.e. how likely STRING judges an interaction to be true, given the available evidence. All scores rank from 0 to 1, with 1 being the highest possible confidence. A score of 0.5 would indicate that roughly every second interaction might be erroneous (i.e., a false positive).

After performing protein-protein interactions from STRING DB , we obtained some uncharacterized proteins using the computational tool STRING, which provided some information. some uncharacteristic proteins

CHI1S2IYG5,CHI1S2JQZ4,PPS1S2JTA0,GPA1S2JAC5,GPA1S2JRE4,COTHS2JW22,COTHS2JKJ7, COTHS2JJH9,COTHS2ITM4,SSF1S2JNV3,HSS1S2J841,GPR78, S2JPS4 With the help of UniProt, we studied the 3D structure of those uncharacterized proteins and some of the interactions between them and other proteins.

The DeepLoc-1.0 server predicts the subcellular localization of eukaryotics proteins using Neural Networks algorithm trained on UniProt proteins with experimental evidence of subcellular localization. It only uses the sequence information to perform the prediction.The DeepLoc-1.0 server requires protein sequence(s) in fasta format, and cannot handle nucleic acid sequences.Paste protein sequence(s) in fasta format or upload a fasta file. After the server successfully finishes the job, a summary page shows up. If an error happens during the prediction a log will appear specifying the error. Use the navigation bar to flip through the various output pages.

Significance of the Findings

This study's integrative approach has highlighted not only established virulence proteins but also a subset of novel, uncharacterized proteins with potential biological significance. The centrality of these proteins in the PPI network, their predicted domains, and cellular localization collectively strengthen their candidacy as putative drug targets. These findings contribute to the growing knowledge of Mucor circinelloides biology and provide a foundation for future functional validation studies.

Conclusion

In conclusion, the increase in Mucorcircinelloides fungal infections that result in mucormycosis poses a serious health risk, especially given the current antifungal treatments' limited effectiveness and possible toxicity. The urgent need for new treatment alternatives is highlighted by the development of resistance to azoles, the main medications used to treat mucormycosis. Furthermore, finding novel therapeutic targets is essential to successfully tackling this issue. This work took a multipronged strategy, using databases and bioinformatics techniques to anticipate key pathogenic genes linked to infection with Mucorcircinelloides. Uncharacterized proteins were found by combining software, research publications, and protein-protein interaction studies made possible by NCBI, UniProt, and STRING DATABASE. Based on protein-protein interactions, we discovered a number of unidentified proteins whose subcellular location and function are yet unknown. CHI1S2IYG5, CHI1S2JQZ4, PPS1S2JTA0, GPA1S2JAC5, GPA1 S2JRE4, COTHS2JW22, COTHS2JKJ7, COTHS2JJH9, COTHS2ITM4, SSF1S2JNV3, HSS1S2J841, GPR78, and S2JPS4 are the novel potential drug targets that we identified by a predictive analysis. Although functional prediction indicates that the majority of the proteins are extracellular and membrane proteins, which suggests good and straightforward targets, these proteins have subcellular localization in the nucleus, cytoplasm, mitochondrion, and golgi.

References

1. Adepu, S., & Ramakrishna, S. (2021). Controlled Drug Delivery Systems: Current Status and Future Directions.Molecules (Basel, Switzerland), 26(19), 5905.
2. Xia X. (2017). Bioinformatics and Drug Discovery.Current topics in medicinal chemistry, 17(15), 1709–1726.
3. Hughes, J. P., Rees, S., Kalindjian, S. B., & Philpott, K. L. (2011). Principles of early drug discovery.British journal of pharmacology, 162(6), 1239–1249.

4. Blanes-Mira, C., Fernández-Aguado, P., de Andrés-López, J., Fernández-Carvajal, A., Ferrer-Montiel, A., &Fernández-Ballester, G. (2022). Comprehensive Survey of Consensus Docking for High-Throughput Virtual Screening.Molecules (Basel, Switzerland), 28(1), 175.
5. Ferreira, L. G., Dos Santos, R. N., Oliva, G., &Andricopulo, A. D. (2015). Molecular docking and structure-based drug design strategies. Molecules (Basel, Switzerland), 20(7), 13384–13421.
6. Aparoy, P., Reddy, K. K., &Reddanna, P. (2012). Structure and ligand based drug design strategies in the development of novel 5- LOX inhibitors. Current medicinal chemistry, 19(22), 3763–3778.
7. Ahmadjian, Vernon , Moore, David and Alexopoulos, Constantine John. "fungus". Encyclopedia Britannica, 26 Mar. 2024, https://www.britannica.com/science/fungus.Accessed 25 April 2024.
8. Moore, D. ,Alexopoulos, . Constantine John and Ahmadjian, . Vernon (2024, March 26). fungus. Encyclopedia Britannica.
9. de Pauw B. E. (2011). What are fungal infections?.Mediterranean journal of hematology and infectious diseases, 3(1), e2011001.
10. Reddy, G. K. K., Padmavathi, A. R., &Nancharaiah, Y. V. (2022). Fungal infections: Pathogenesis, antifungals and alternate treatment approaches. Current research in microbial sciences, 3, 100137.
11. Garber G. (2001). An overview of fungal infections. Drugs, 61 Suppl 1, 1–12.
12. Reddy, G. K. K., Padmavathi, A. R., &Nancharaiah, Y. V. (2022). Fungal infections: Pathogenesis, antifungals and alternate treatment approaches. Current research in microbial sciences, 3, 100137. https://doi.org/10.1016/j.crmicr.2022.100137
13. Hurdeal, V. G., Gentekaki, E., Hyde, K. D., Nguyen, T. T. T., & Lee, H. B. (2021). Novel Mucor species (Mucoromycetes, Mucoraceae) from northern Thailand.MycoKeys, 84, 57–78. https://doi.org/10.3897/mycokeys.84.71530