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Research paper

Bioinformatic survey of ABC transporters in dermatophytes

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ABSTRACT

ATP binding cassette (ABC) transporters constitute a very large and ubiquitous superfamily of membrane proteins. They are responsible for ATP hydrolysis driven translocation of countless substrates. Being a very old and diverse group of proteins present in all organisms they share a common feature, which is the presence of an evolutionary conservative nucleotide binding domain (NBD) — the engine that drives the transport. Another common domain is a transmembrane domain (TMD) which consists of several membrane-spanning helices. This part of protein is substrate-specific, thus it is much more variable. ABC transporters are known for driving drug efflux in many pathogens and cancer cells, therefore they are the subject of extensive studies. There are many examples of conferring a drug resistance phenotype in fungal pathogens by ABC transporters, however, little is known about these proteins in dermatophytes — a group of fungi causing superficial mycoses. So far only a single ABC transporter has been extensively studied in this group of pathogens. We analyzed available genomic sequences of seven dermatophyte species in order to provide an insight into dermatophyte ABC protein inventory. Phylogenetic studies of ABC transporter genes and their products were conducted and included ABC transporters of other fungi. Our results show that each dermatophyte genome studied possesses a great variety of ABC transporter genes. Detailed analysis of selected genes and their products indicates that relatively recent duplication of ABC transporter genes could lead to novel substrate specificity.

1. Introduction

Dermatophytes are a highly specialized group of keratinophilic fungi that are responsible for superficial mycoses of humans and animals (Weitzmann, 1995). Although not fatal, dermatophyte infections are a major cosmetic problem in otherwise healthy humans worldwide and are difficult to treat. Moreover, in immunocompromised patients they may pose a serious threat. Traditionally dermatophytes are divided into three genera: *Microsporum*, *Trichophyton* and *Epidermophyton*, although the exact taxonomic relations of this fungal group are far from being fully resolved (Gräser, Scott and Summerbell, 2008). Despite the common occurrence of dermatophyte infections relatively little is known about molecular biology of these pathogens. This is partially caused by the fact that dermatophytes are less amenable to experimental procedures compared to other more relevant fungal pathogens and there are no well-developed and easy to use tools for genetic modifications (White et al., 2008). Recently completed sequencing projects of a few dermatophyte species provide an insight into genetics of this fungal group (Burmester et al., 2011; Martinez et al., 2012).

ATP-binding cassette (ABC) proteins are one of the largest protein superfamilies, containing more than three thousand members found in all living organisms (Jungwirth and Kuchler, 2006). As the name suggests, these proteins bind and hydrolyze ATP while playing a variety of roles. The best known members of this group are transmembrane transporters containing ABC, called ABC transporters.

ABC transporters are the largest group of all transporters present in cells (Rees et al., 2009). These proteins utilize energy from hydrolysis of ATP to translocate an extremely broad range of substrates from small inorganic ions to large molecules including toxins. ABC transporters are responsible for drug resistance of a myriad of pathogens both prokaryotes and eukaryotes. Human ABC transporters are heavily investigated due to their involvement in drug resistance of cancer cells (Deeley, 2006). Moreover, malfunction of ABC genes is a cause of a variety of human diseases including cystic fibrosis and Tangier disease (Dean et al., 2011).

Despite their old evolutionary age, all ABC transporters share a similar structure with hallmark domain organization (Fig. 1). At least one nucleotide binding domain (NBD) is present and accompanied by several transmembrane helices, which are organized in a so called transmembrane domain (TMD). A typical ABC transporter is composed of two NBDs and two TMDs arranged in forward $(TMD–NBD)_2$ or reverse $(NBD–TMD)_2$ configuration (Fig. 1b).

Abbreviations: ABC, ATP binding cassette; NBD, nucleotide binding domain; TMD, transmembrane domain.

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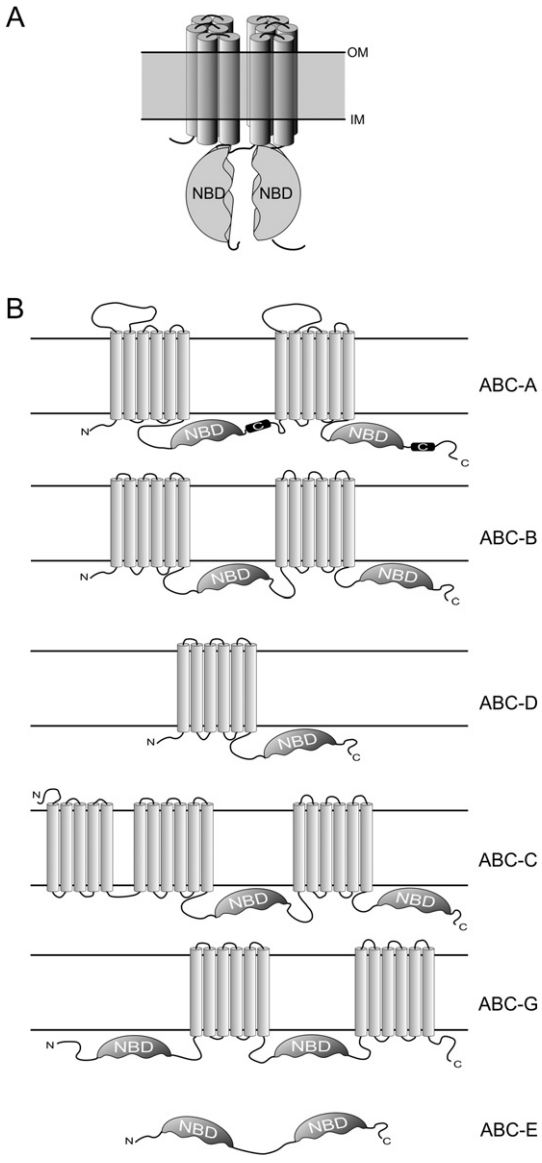

Fig. 1. A) Schematic structure of ABC transporters. B) Topology of ABC transporter subfamilies.

However, half-size transporters as well as other structure modifications are also present. The main role of NBDs of all ABC transporters is to catalyze ATP hydrolysis and this part of transporters is more evolutionarily stable (Hollenstein et al., 2007). On the other hand TMDs form a channel through a lipid membrane (Fig. 1a) and are responsible for translocation of a substrate (Seeger and van Veen, 2009). A variety of ABC transporter substrates are reflected by a divergence of TMD sequences. Based on the sequence similarity and domain organization, human ABC transporters are divided into eight subfamilies A to H (Glavinas et al., 2004; Verrier et al., 2008). This nomenclature has been adopted for most eukaryotic ABC transporters.

ABC transporters can be divided into two major subtypes: importers and exporters, although importers seem to be present mainly in prokaryotes but also in plants (Rice et al., 2014).

Previous studies of fungal ABC transporters indicate their important role in pathogenicity and drug resistance (Coleman, Mylonakis and Finlay, 2009), but in the case of dermatophytes, this knowledge is limited. Here we provide the first survey of ABC transporter genes present in dermatophyte genomes with a detailed analysis of selected ABC-B transporters.

2. Material and methods

2.1. Genomic sequences

Genomic sequences of seven dermatophytes were used in the research. Five of them: zoophilic *Microsporum canis* (GenBank accession number ABVF00000000), and *Trichophyton equinum* (ABWI00000000), geophilic *Microsporum gypseum* (ABQE00000000) as well as anthropophilic *Trichophyton rubrum* (ACPH01000000), and *Trichophyton tonsurans* (ACPI00000000) were downloaded from Dermatophyte Comparative Sequencing Project, Broad Institute of Harvard and MIT website (http://www.broadinstitute.org). An additional two, both from zoophilic species: *Arthroderma benhamiae* (ABSU00000000) – a teleomorphic form of *Trichophyton mentagrophytes* – and *Trichophyton verrucosum* (ACYE00000000), were obtained from GenBank (Burmester et al., 2011).

2.2. Gene sequence prediction and identification of ABC-MDR genes

Gene sequences predicted using GeneMark.hmm software (Lomsadze, 2005), as well as gene sequences downloaded from Broad Institute website (http://www.broadinstitute.org) were included in the study. In further studies sequences of coding regions with introns (referred to as genes), joint exons and amino acid sequences were used.

ABC transporters and related proteins were searched within obtained amino acid sequences using hmmsearch (Ter-Hovhannisyan et al., 2008) using Hidden Markov Model (HMM) of a nucleotide binding domain (NBD) from Pfam database (PF00005) (Finn et al., 2013) at E value threshold = 1e−15. In the next step the identified NBD-containing protein was searched against a transmembrane domain HMM (TMD; Pfam PF00664 and PF12698). In order to identify additional domains in selected protein searches against entire Pfam and Conserved Domain Database (CDD) databases were performed.

Transmembrane helices of TMD were predicted with HMMTOP (Tusnady and Simon, 2001). NBDs were pre-localized in cytoplasm in order to improve prediction accuracy. Boundaries of transmembrane domains were corrected according to the position of predicted transmembrane helices.

In order to detect any unpredicted genes, we searched raw genomic sequences using tblastn (NCBI BLAST). Before the search the sequences of all identified ABC transporter genes were masked from genomic sequences. In the next step, all amino acid sequences of previously identified ABC transporters were used as queries in tblastn search. This approach made it possible to detect up to three additional genes per species.

2.3. ABC transporter gene characterization

All sequence alignments were performed with T-coffee (Notredame et al., 2000) and ClustalX (Larkin et al., 2007). Th full length of domains of ABC transporter genes were determined using Conserved Domain Database (CDD) (Marchler-Bauer and Bryant, 2004).

Apart from Walker A (GxxGxGKST) and B (four aliphatic residues followed by 2 negatively charged residues, typically ILLLDE or VLLLDE), other motifs within the nucleotide binding domain were determined such as A loop (conservative tyrosine located ~25 amino acid residues upstream of walker A), D loop (invariant aspartic acid residues), signature motif (LSGGQ), and X-loop (TxVGExG). GenBank searches of nucleotide sequences of coding regions and amino acid sequences of protein products were performed also in non-dermatophyte species in order to find sequences similar to the ones identified by us, and best matches were chosen for further analyses.

Similarity charts present the percentages of identical amino acid positions within each subfamily with a window width of 100 amino acids. In order to obtain an equal length of sequences, alignments were truncated at the ends, and large insertions were cut. Mean similarity value was calculated as an overall percentage of similar amino acid sequences.