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Short Report

Molecular Characterization of a *Squalene epoxidase* Gene in Dermatophyte Pathogen *Trichophyton tonsurans*

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ABSTRACT

Background: *Trichophyton tonsurans* is one of the dermatophyte fungi which invades the skin and hair of human. Several properties of this fungus have been investigated so far. However a few studies were carried out in the field of molecular biology of this fungus. In the present study, we tried to identify the *Squalene epoxidase* gene which is related to synthesis of ergosterol in this fungus. **Methods:** Pairs of 23 and 24 nucleotides primers were designed from highly conserved regions of the similar genes in other fungi. Mentioned primers were utilized in PCR by using isolated genomic DNA of *T. tonsurans* whereas the PCR fragments were then sequenced. **Results and Conclusion:** Nucleotides (n = 558) have been sequenced from this new gene which encodes a polypeptide with 186 amino acids. Sequences comparison in gene data banks (NCBI, NIH) for this part of DNA and its deduced amino acid revealed significant homology with members of the eukaryotic *Squalene epoxidase*. *Iran. Biomed. J. 12 (1): 55-58, 2008*

Keywords: Dermatophyte, *Trichophyton tonsurans*, *Squalene epoxidase*, Ergosterol, PCR

INTRODUCTION

Eukaryotic and prokaryotic cells have a membrane that isolates them from environment. The component of cell membrane is very important, because of contributing in survival of cell. Sterols are one of the necessary compounds in most eukaryotic cells but fungi have a unique kind of sterol named ergosterol. This compound is important in different actions such as regulation of cycle cell, enzyme activation and permeability [1].

Squalene epoxidase is a microsomal membrane-associated enzyme that acts as an important regulator in the sterol biosynthetic pathway. Ergosterol contributes to photooxidation in fungi. This enzyme needs oxygen, Nicotine amide Adenine Dinucleotide Phosphate Hydrogen) and Flavin Adenine Dinucleotide for activation [2].

In yeasts, *Squalene epoxidase* makes correspondence between lipid particles and endoplasmic reticulum [3]. The main target of some antifungal drugs is the mentioned enzyme. In recent studies, it has been proved that *Trichophyton rubrum* and *Aspergillus fumigatus* are resistant to terbinafine [4, 5]. Dermathophytes are filamentous fungi that are keratinophilic and invade the skin and hair of human. *Trichophyton tonsurans* is responsible for the vast majority of dermatophyte infections [6-8]. This fungus is an important agent of tinea gladiatorum [9]. In this study, the molecular identification of *Squalene epoxidase* gene in *T. tonsurans* was the main aim.

MATERIALS AND METHODS

Fungal Specimen Preparation. In order to perform this fundamental study, sampling was done

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from patients suspicious to dermatophytosis in Mycology Laboratory at School of Public Health, Tehran University of Medical Sciences (Tehran, Iran) in 2006. After macroscopical and microscopical examinations, the existence of *T. tonsurans* was proved.

DNA isolation. High molecular weight DNA from *T. tonsurans* was isolated by modified method of Choi *et al*. [10] Briefly, the harvested mycelial mass was flash-frozen in liquid nitrogen and ground to a fine powder in a porcelain mortar. The mycelial powder was suspended in DNA extraction buffer containing: Tris-HCl (pH 8.0), EDTA, SDS, and proteinase-K. The suspension was then incubated at 65°C for 1 hour and the cellular debris was removed by centrifugation at 3000 ×g for 15 min. After extracted once with phenol-chloroform-isoamyl alcohol and once with chloroform-isoamyl alcohol, the DNA was precipitated by addition of an equal volume of ethanol and resuspended in distilled water.

PCR analysis. PCR analysis of genomic DNA was performed according to the standard protocol using synthetic oligonucleotide primers. Briefly, 2.5 µl of each primer (OD 20) was added to a volume of 45 µl containing: 10 × PCR buffer + $MgCl_2$, 10 mM dNTP mix, 1 unit/µl of Taq DNA polymerase and template (genomic DNA). The PCR program employed for amplification of the appropriate part of this gene was 94°C for 1 min, 52°C for 1 min, and 72°C for 1 min and 30 s; whereas the program was repeated 35 cycles. PCR products were analysed by electrophoresis through a 1% agarose gel.

Purification and sequencing of PCR products. The fragment deduced by PCR amplification should be purified in order to eliminate waste materials. For this purpose, desired fragment was cut and purified by Qiagen Kit (Qiagen Company, Germany) Sequencing of the PCR fragments. After purification of PCR fragments by Qiagen Kit, sequencing of the genomic DNA was performed with the Dye Terminator Cycle Sequencing Kit (MWG Biotech, Germany), by using the isolated double-stranded DNA as template and synthetic oligomeric internal primers were designed according to the highly conserved regions from other fungi. Sequencing of each part was repeated. The nucleotide sequence of DNA was compared with the counter parts in gene data bank (NCBI, NIH).

RESULTS AND DISCUSSION

Isolation and characterization of a Squalene epoxidase gene from T. tonsurans. After amplification of a part of this gene by PCR, it has been identified as a new *Squalene epoxidase* gene in dermatophyte pathogen *T. tonsurans*. The approximate length of this new gene was 600 bp according to the comparison with size marker (Fig. 1).

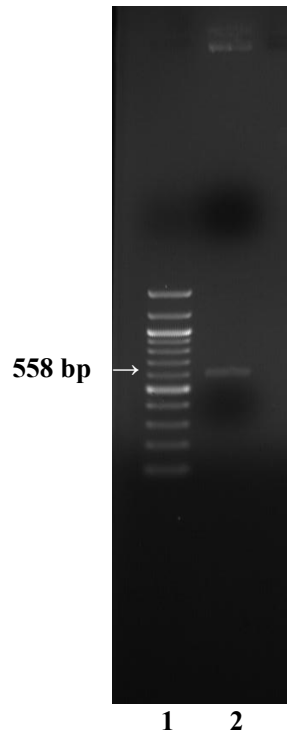

Fig. 1. PCR product deduced from a part of *Squalene epoxidase* gene in *T. tonsurans* (lane 2). The length of size marker bands (lane 1) is 1300, 1200, 1100, 1000, 900, 800, 700, 600, 500, 400, 300, 200 and 100 bp (from top to bottom).

Sequencing analysis after amplification by PCR revealed that this part of new gene has 558 nucleotides which encode a polypeptide with 186 amino acids. The partial nucleotide and amino acid sequences of this new gene (*T. tonsurans Squalene epoxidase* gene) are presented in Figure 2.

Comparison in gene data banks (NCBI, NIH) for both the nucleotide sequence of DNA and its deduced amino acid revealed a significant homology with members of the eukaryotic *Squalene epoxidae.*

The amino acid sequence of the encoded protein was about 95% identical to that obtained from *T. rubrum*, 75% to *Emericella nidulans*, 74% to

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  1 P F P S D A N D K I P E G R C F H H G R  20
  1 cca ttc ccg agc gac gcg aac gat aaa att ccc gaa ggc agg tgc ttc cac cat ggc cgg  60

 21 F I M R L R E A A M A N P N V T I V E T  40
 61 ttt att atg cgc ctt cga gag gcg gca atg gca aac ccc aac gtc acg ata gtc gag acc 120

 41 K A V D T I K S T H T G E V L G V Q C L  60
121 aag gcg gtt gac acc ata aag tcc acg cat acc ggg gag gtc ctt gga gtc cag tgc ctt 180

 61 T D G K K D F Y F G P L T V V A D G Y A  80
181 aca gat ggc aag aag gac ttt tat ttt ggg ccg ctg acg gtt gtc gcc gat ggt tat gca 240

 81 S T F R K E Y L P I T P V A K S K F W G 100
241 tct acc ttc cga aag gaa tat ctc ccg ata acg ccg gtc gcg aaa tcg aaa ttc tgg ggc 300

101 L E L I D A K L P I P G H G H V V L G D 120
301 ctg gag ctt ata gat gcc aag tta ccg ata cca ggc cac ggc cat gtt gtc ctg ggt gat 360

121 F P P I L I Y Q I G E H E T R I L I D I 140
361 ttc cca ccg att ttg ata tac cag att gga gag cat gag acg cga atc cta att gat ata 420

141 P D N L P S A S V A N G G V K G H M R N 160
421 ccg gac aac ctg ccg tcg gcc tca gtt gcg aac gga ggt gta aaa ggt cac atg cgg aat 480

161 V V L P S L P E C I R P S F A A A L E K  180
481 gtc gtc ctt ccg tct ttg cca gag tgt att cgg ccg tca ttt gcg gca gcc ttg gag aag  540

181 G G L R S M  186
541 gga ggg ctc cgg tca atg  558
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Fig. 2. Partial nucleotide and its deduced amino acid sequences of *Trichophyton tonsurans Squalene epoxidase* gene.

A. fumigatus and 65% to *Aspergillus niger*. The amino acid composition of this protein indicates that it is rich in glycine (9.13%) and leucine (8.6%); in contrast, the amounts of tryptophan (0.53%) and glutamine (1.07%) were poor.

In the present study, we report the identification and molecular characterization of the *T. tonsurans* gene encoding a *Squalene epoxidase* enzyme. Generally, most of the molecular investigations have been carried out on *Trichophyton* and *Microsporum* among three genera of dermatophytes. *T. tonsurans* is an anthropophilic pathogen which causes *tinea capitis*, *tinea corporis* and *tinea unguium* and considered as an important causative agent of dermatophytosis in human. According to the involvement of this pathogen in human infections, we decided to survey about one important molecular aspects of this fungus which probably influences its pathogenicity and resistance to antifungal drugs. Less molecular information is available about this fungus; however there are reports on genome sequencing of this fungus which has medium density. There are some surveys on internal transcribed spacer (ITS) sequencing, phylogeny of this fungus and similarity of this dermatophyte to other fungi [11]. A few restriction enzymes such as *Mva*I and *Hinf* I have been used for rDNA and ITS regions of mentioned fungus [12]. It is noteworthy that some molecular diagnosis methods i.e. RAPD, RFLP and DNA-fingerprinting were used in dermatophytes identification [13, 14]. *Squalene epoxidase* is associated with ergosterol synthesis which is one of the membrane components influences on permeability, phothooxidation and activation of enzymes [1]. This enzyme has been detected in *T. rubrum*, *A. fumigatus*, *Saccharomyces cerevisiae*, *Candida albicans*, *Neurospora crassa* and human [2, 5, 8, 15-17].

Comparison analysis of this new gene sequence and its deduced protein revealed a significant homology with other fungi e.g. 95% with *T. rubrum*, 75% with *E. nidulans*, 74% with *A. fumigatus* and 65% with *A. niger*. This is obvious that the maximum and minimum amounts of amino acids are the same among these proteins. Our studies reveals a significant similarity with those of Osborne *et al*. [5] on *T. rubrum* as leucine and glycine were major amino acids and tryptophan was minor [5]. In the Rocha *et al*. [18] survey, leucine and alanine were major whereas tryptophan and cysteine were in the

least. Gracia *et al.* [NCBI, NIH; Accession No: AY619002] concluded that *A. fumigatus Squalene epoxidase* was rich in leucine and valine whereas amounts of tryptophan, cysteine and methionine were poor which reveals a significant consistency with the present survey. Dave *et al.* [NCBI, NIH; Accession No: AJ748126] proved that in the *Squalene epoxidase* of *A. niger*, there is a great homology in amino acids amounts with *T. tonsurans*.

It can be concluded that there is a great similarity between nucleotide sequence of *Squalene epoxidase* gene and its deduced amino acids of *T. tonsurans* with other fungi and eukaryotes. It is desired to design new fungal drugs by further investigation on this new gene.

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REFERENCES

1. Trigos, A. (2002) Selective destruction of microscopic fungi through photooxidation of ergosterol. *Mycologia 94 (4): 563-568.*
2. Ruchenstubl, C., Eidenberger, A., Lang, S. and Turnowsky, F. (2005) Single amino acid exchanges in FAD-binding domains of *Squalene epoxidase* of *Saccharomyces cerevisiae* lead to either loss of functionality or terbinafine sensitivity. *Biochem. Soc. Trans. 33 (5): 1197-1201.*
3. Regina, L., Karina, L., Erwin, Z., Horst, A., Armin, S., Sepp, D.K., Friederike, T. and Gunther, D. (1998) Dual localization of *Squalane epoxidase* Erglp in yeast reflects a relationship between the endoplasmic reticulum and lipid particles. *Mol. Biol. Cell 2: 375-386.*
4. Liu, W., Greogory, S.M., Michail, S.L., Russel, E.L. and Dimitrios, P.K. (2004) Extra copies of the *Aspergillus fumigatus Squalene epoxidase* gene confer resistance to terbinafine. Genetic approach to studying gene dose-dependent resistance to antifungals in *A. fumigatus*. *Antimicrob. Agents Chemother. 48 (7): 2490-2496.*
5. Obsorne, C., Ingrid, L., Bertrand, F. and Neils, R. (2005) Amino acid substitution in *Trichophyton rubrum Squalene epoxidase* associated with resistance to terbinafine. *Antimicrob. Agents Chemother. 49 (7): 2840-2844.*
6. Fullerl, C., Child, F.J., Midgley, G. and Hinggins, E.M. (2003) Diagnosis and management of scalp ring worm. *BMJ 326 (7388): 539-541.*
7. Gupta, A.K. and Summerbell, R.C. (1998) Increased incidence of *Trichophyton tonsurans* tinea capitis in Ontario, Canada between 1985 and 1996. *Med. Mycol. 36: 55*-60
8. Poisson, D.M., Rousseau, D., Defo, D. and Esteve, E. (2005) Outbreak of tinea corporis gladiatorum, a fungal skin infection due to *Trichophyton tonsurans* in a French high level judo team. *Euro Surveill. 10 (9): 187-190.*
9. Kasai, T. (2005) Epidemiological survey of *Trichophyton tonsurans* infection in Tohoku district and its clinical problems. *Nippon Ishinkin Gakkai Zasshi 46 (2): 87-91.*
10. Choi,G.H., Marek, E.T., Schardl, C.L., Richey, M.G., Chang, S. and Smith, D.A. (1990) A stress-responsive gene in *Fusarium* spp. *J. Bacteriol. 172: 4522-4528.*
11. Woodgyer, A. (2004) The curious adventures of *Trichophyton equinum* in the realm of molecular biology a modern fairy tale. *Med. Mycol. 42 (5): 397-403.*
12. Mochizuki, T., Tanabe, H., Kavasaki, M., Ishizaki, H. and Jack, S. (2003) Rapid identification of *Trichophyton tonsurans* by PCR-RFLP analysis of ribosomal DNA regions. *J. Dermatol. Sci. 32 (1): 25-32.*
13. Graser, Y., Kuijpers, A.F.A., Presber, W. and DeHoog, G.S. (1999) Molecular taxonomy of *Trichophyton mentagrophytes* and *T. tonsurans*. *Med. Mycol. 37: 315-330.*
14. Jeong, A.K., Kayoko, T., Kazutaka, F., Kazuko, N. and Makoto, M. (1999) Identification and genetic homogeneity of *Trichophyton tonsurans* isolated from several regions by random amplified polymorphic DNA. *Mycopathologia 145:1-6.*
15. Jahnke, L. and Klein, H.P. (1983) Oxygen requirements for formation and activity of the *Squalene epoxidase* in *Saccharomyces cerevisiae*. *J. Bacteriol. 155 (2): 488-492.*
16. Pichler, H., Gaigg, B., Hrastnik, C., Achleitner, G., Kohlwein, S.D., Zellnig, G., Perktold, A. and Daum, G. (2001) A subfraction of the yeast endoplasmic reticulum associates with the plasma membrane and has a high capacity to synthesize lipids. *Eur. J. Biochem. 268 (8): 2351-2361.*
17. Satoh, T., Horie, M., Watanabe, H., Tsuchiya, Y. and Kamei, T. (1993) Enzymatic properties of *Squalene epoxidase* from *Saccharomyces cerevisiae*. *Biol. Pharm. Bull. 16 (4): 349-352.*
18. Rocha, E.M., Gardiner, R.E., Park, S., Martinez-Rossi, N.M. and Perlin, D.S. (2006) A Phe 389 Leu substitution in ErgA confers terbinafine resistance in *Aspergillus fumigatus*. *Antimicob. Agents Chemother. 50 (7): 2533-2536.*