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Decreased expression of *Malassezia furfur* virulence factors after Q-switched Nd:YAG laser irradiation

Background: Malassezia spp. are lipophilic yeasts implicated in the pathogenesis of chronic skin diseases. Repeated therapies are often necessary due to the recurrence of this type of disease. Recently, laser and light-based devices used for the treatment of some skin diseases have shown good efficacy, few contraindications, and minimal side effects. The neodymium-doped yttrium aluminium garnet (Q-switched Nd:YAG) laser is one of the most commonly used lasers in dermatology. *Objectives:* The aim of this study was to evaluate the effect of the Q-switched Nd:YAG laser (Medlite C6 laser, Conbio, USA) on the pathogenic mechanisms of *M. furfur* during skin infections. *Materials & Methods:* Following laser exposure, the ability of *M. furfur* to retain phospholipase activity, upregulate the aryl receptor and its associated pathway, and stimulate the immune response were tested. *Results:* The Q-switched Nd:YAG laser was shown to attenuate the virulence of *M. furfur. Conclusion:* The Q-switched Nd:YAG laser should be considered as a valid therapeutic alternative for the treatment of *Malassezia*-associated infections.

Key words: Malassezia, Q-switched Nd:YAG Laser, skin, aryl hydrocarbon receptor (AhR), beta-defensin 2

Malassezia is a genus of lipid-dependent yeasts that includes 14 species, with a pathogenic role in common recurrent skin diseases, among which one of the most frequently isolated is *M. furfur*.
Among their virulence factors, *Malassezia* produce various indole ligands of the aryl hydrocarbon receptor (AhR) [1]. AhR interacts also with transcriptional factor NF-κB and strongly modulates its actions [2], leading to modification of the inflammatory response [3]. Phospholipase activity is a virulence factor reported in some strains of *Malassezia* spp. [4], which leads to degradation of intercellular lipid of the stratum corneum, resulting in its dysfunction and exfoliation. *M. furfur* has been shown to strongly upregulate the expression of antimicrobial peptide beta-defensin-2 (HBD-2) in human keratinocytes. [5].
The neodymium-doped yttrium aluminium garnet (Nd:YAG) laser is one of the most commonly used lasers in dermatology. The laser produces various wavelengths and can operate in continuous, long-pulsed, Q-switched, or potassium titanyl phosphate (KTP) modes [6]. The Q-switched 1064-nm Nd:YAG has many therapeutic indications, including onychomycosis [7], and was recently demonstrated *in vitro* to reduce inflammation and promote cytoprotection in HaCat cells during *Candida albicans* infection [8].
Since the effectiveness of photodynamic therapy for the treatment of *Malassezia* infection has already been tested [9], the aim of this study was to evaluate the ability of the Q-switched Nd:YAG laser to interfere with pathogenic mechanisms of *M. furfur.*

Materials and methods

The strain of *M. furfur* ATCC® 14521 was cultured as previously described [5].
For the phospholipase activity assay, *M. furfur* was irradiated with a 1064-nm Q-switched Nd:YAG laser (Medlite C6 laser, Conbio, USA) at a fluence of 8 J/cm^2, a pulse width of 5 ns, and a spot size of 4 mm. The irradiation was performed over the entire surface of the agar plate twice, at an interval of 1 second. The irradiated and non-irradiated *M. furfur* was cultured on SDA with 10 mM β-endorphin at 30 °C for 10 days, and colonies were then transferred to egg-yolk agar and incubated for 10 days at 30 °C.
HaCat cell lines (Elabscience) were cultured as previously described [5] and infected at 80% confluence with the non-irradiated and irradiated *M. furfur* (30:1; yeast/cell) for 24 hours. After infection, total mRNA was extracted and real-time PCR for *AhR*, *CyP1A1*, *CyP1B1*, and *hBD-2* was carried out with the LC Fast Start DNA Master SYBR Green kit [10] (Roche Diagnostic) (*figure 1A*).
The presence of hBD-2 was also analysed in cell supernatants by ELISA assay (Elabscience).

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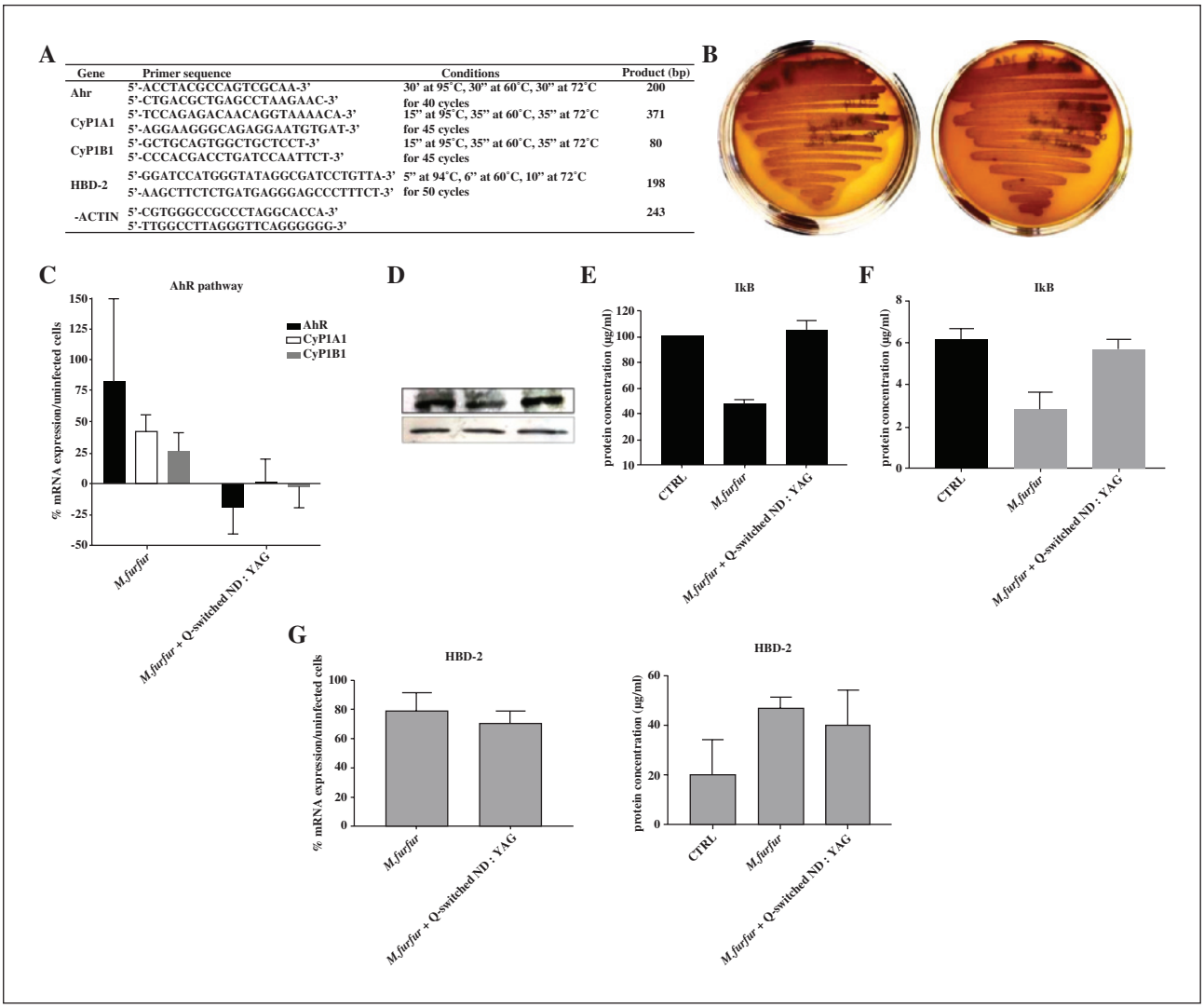

Figure 1. **A**) Primer sequences and amplification programs. **B**) Phospholipase activity in unirradiated and irradiated *M. furfur.* **C**) Effect of the Nd:YAG laser on activation of the AhR receptor in HaCat cells infected with *M. furfur* based on real-time PCR. Values of mean ± SD are expressed as percentage of relative mRNA expressed in each group, compared to uninfected cells (ctrl). **D**, **E**) Western blot analysis for IκB in HaCat cells infected with *M. furfur* and/or irradiated with Nd:YAG laser for 48 hours (β-tubulin was used as an internal control for protein loading). **F**) IkB in cellular supernatants based on ELISA. **G**) Effects of the Nd:YAG laser on the expression of HBD-2 in HaCat cells infected with *M. furfur* based on real-time PCR and ELISA. Values of mean ± SD are expressed as percentage of the expression of relative mRNA in each group, compared to uninfected cells (ctrl).

IkB-alpha expression was evaluated in cell lysates and supernatants by western blot [5] (Santa Cruz Biotechnology, Inc.) and ELISA assay (Elabscience), according to the manufacturers' instructions.

Significant differences among groups were assessed through two-way ANOVA using GraphPad Prism 6.0. The data were expressed as mean ± standard deviation (SD) of three independent experiments.

Results and discussion

Phospholipase activity is revealed by the appearance of a precipitation halo around the colonies, due to the release of calcium and fatty acids by the degradation of the phospholipids in the egg yolk. Our results show that laser irradiation inhibits phospholipase activity of *M. furfur*, thus limiting penetration of the pathogen (*figure 1B*).

Analysis of the expression of *AhR* and cytochromes *CyP1A1* and *CyP1B1* (closely related to the activation of AhR) show that the activation of these genes, induced by *M. furfur* infection in HaCat cells, was drastically reduced by the preliminary laser treatment (*figure 1C*).

During *M. furfur* infection, the activation and subsequent translocation of Nf-κB in the nucleus leads to a decrease in the concentration of the IκB protein in the cytoplasm. Our results reveal that treatment with Nd:YAG completely reverses this trend, indicating an inhibition of this transcriptional pathway (*figure 1D-F*).

However, the Q-switched Nd:YAG laser does not affect the production of HBD-2 by keratinocytes, thus retaining

the host's ability to activate the immune and antimicrobial response (*figure 1G*).

In conclusion, the present study provides previously undocumented evidence that the use of the Q-switched Nd:YAG laser can be a valid therapeutic alternative for skin diseases caused by *M. furfur* infections, either alone or in combination with conventional therapies. Clinical trials are necessary to further confirm the efficacy and safety of this therapy. ■

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