Differentiation by random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) of Candida albicans isolated from upper respiratory tract in patients with non-small cell lung cancer*

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Cancer patients are predisposed to fungal infections caused by Candida albicans, especially to oral or respiratory tract candidiasis. The aim of this study was to estimate genetic diversity by RAPD-PCR (random amplified polymorphic DNA-polymerase chain reaction) of C. albicans isolated from upper respiratory tract of 100 patients with non-small cell lung cancer. Among 52 strains, 34 genotypes were defined. 10 clusters comprising 28 (53.85%) isolates with similarity coefficient ≥ 80% were formed. The remaining 24 (46.15%) isolates represented individual genotypes. The RAPD-PCR technique revealed genomic variability within C. albicans isolated from upper respiratory tract of the cancer patients.

Key words: RAPD-PCR, Candida albicans, upper respiratory tract, genetic diversity

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INTRODUCTION

Yeasts belonging to Candida spp., mainly Candida albicans, are commensal organisms that can be isolated from the gastrointestinal tract, as well as oral and vaginal mucosa of many, if not all, healthy individuals (Kim & Sudbery, 2011). At the same time, C. albicans is one of the most common opportunistic pathogens which can cause endogenous infections from superficial to seriously deep-seated mycoses under altered host conditions (Chong et al., 2003; Pires-Gonçalves et al., 2007; Costa et al., 2008; Baccelo et al., 2010; Gültekin et al., 2011).

The number of infections caused by these microorganisms has grown rapidly in the last years and they are an important medical problem in patients from high risk groups, such as immunocompromised people or malignant cancer patients. Anticancer treatment including chemotherapy and/or radiotherapy is still associated with several long-term adverse effects. Among them, oral candidiasis is one of the major sources of illness, although strict oral hygienic care by using antifungal reagents is being employed to prevent it (Teanpaisan et al., 2008; Sun et al., 2009; Lee et al., 2010).

Cancer patients, among them patients with non-small cell lung cancer, are also predisposed to infections caused by C. albicans, especially oral or respiratory tract candidiasis. Such infections are usually endogenous in origin due to colonization of the upper respiratory tract mucosa by the yeast. There are many important risk factors predisposing patients to such colonization, including e.g.: long-term anticancer therapy (chemotherapy and/or radiotherapy), long hospital stay, surgical procedures, intravenous drug administration or endocrinological disorders (Chong et al., 2003; Samaranayake et al., 2003; Costa et al., 2008).

Since the colonizing and invading Candida sp. isolates are usually the same, actual knowledge about prevalence, diversity and phenotypic or genotypic characteristics of the isolates from the upper respiratory tract may be applied for epidemiological purposes. Recent advances in molecular techniques have generated several typing methods based on PCR for genetic assessment of genetic relatedness of bacterial or fungal strains. One of them, RAPD-PCR (random amplified polymorphic DNA-polymerase chain reaction) analysis is a useful discriminatory method of Candida spp. in clinical practice (Saran et al., 2008; Krawczyk et al., 2009; Baccelo et al., 2010; Gültekin et al., 2011; Samaranayake et al., 2011).

The aim of this study was to estimate by RAPD-PCR the genetic diversity of C. albicans isolated from upper respiratory tract of patients with non-small cell lung cancer.

MATERIALS AND METHODS

The study group of patients. The study enrolled 100 patients with non-small cell lung cancer (NSCLC), hospitalized at the Department of Thoracic Surgery of Medical University of Lublin. The study population consisted of 87 males and 13 females with the average age of 62 years (range 37–73) from South-East Poland. These patients were diagnosed with ca. macrocellulare, ca. planoepitheliale, ca. planoeptiheliale partim macrocellulare, ca. macrocellulare partim planoeptiheliale and adenocarcinoma. Some patients were after pre- or post-operative chemotherapy including cis-platina and Vepesid, administered in doses according to the standard procedures. None of the patients had lesions in oral cavity. The Ethical Committee of the Medical University of Lublin approved the study protocol (No. KE-0254/75/2011).

Microbiological cultures. Specimens were obtained from throat and nostrils with sterile alginate-tipped swabs. The specimens were microscopically examined for the presence of yeast organisms and then streaked onto Sabouraud’s dextrose agar, amphotericin B, and chloramphenicol at 25°C for 24 h.

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Abbreviations: RAPD-PCR, random amplified polymorphic DNA-polymerase chain reaction

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swabs on aluminium shafts. Within one hour, the swabs were plated on Sabouroud agar with chloramphenicol and incubated aerobically at 35–38°C for 48 h. Yeast were identified by colony morphology, Gram staining and a biochemical method (API Candida, API AUX BioMerieux).

**RAPD-PCR for *C. albicans* differentiation.** The DNA from the isolates was prepared using GeneMATRIX Yeast DNA Purification Kit (EURx) according to the manufacturer’s procedure. RAPD-PCR method was performed with RSD12 primer (Samaranayake et al., 2003; Pinto et al., 2004; Pires-Gonçalves et al., 2007; Waltimo et al., 2001). PCR reactions were carried out in a thermocycler and amplification conditions were as follows: 40 cycles of denaturation at 94°C for 30 s, primer annealing at 57°C for 2 min and elongation at 72°C for 2 min. The final elongation at 72°C was extended to last 15 min. The polymerase chain reactions were performed in 0.5 ml microcentrifuge tubes in a final reaction mixture containing 100–400 ng of *C. albicans* DNA as template, 1xPCR buffer for Taq DNA polymerase (Fermentas), 1 u/µl of Taq DNA polymerase (Fermentas), 200 µM dNTPs (Fermentas), 2.5 mM MgCl₂ (Fermentas), 1.25 µM of primer RSD 12 (Proligo primer&probes).

For each experiment, the size of DNA fragments amplified by PCR was determined by direct comparison with the DNA marker — 100 bp Ladder Plus (Fermentas). Control tubes without template DNA were included in each run and reproducibility was checked for each reaction. The PCR products were separated using electrophoresis in agarose gels (1.5%) at 120 V for approximately 100 min at room temperature in TBE buffer (Tris Borate Electrophoretic Buffer, 89 mMTris/HCl, 89 mM boric acid, 2.5 mM EDTA, pH 8.0) (Sigma). Reaction products were detected by ethidium bromide and visualized with UV light.

**In silico analysis.** Different banding positions of RAPD fingerprinting patterns of *C. albicans* isolates were analyzed using the BioGene program (Polygen). For the analysis of relationships among the strains with this software, dendrograms are constructed by the unweighted pair group method. Each lane pattern was compared to every other pattern through computations of a similarity coefficient, which compares the band positions.

**RESULTS**

A total of 52 isolates of *C. albicans* colonizing mucosal membranes of the upper respiratory tract were obtained from NSCLC patients. Phenotypic differentiation was carried out using a biochemical microtest (API Candida, API AUX, BioMerieux). RAPD-PCR technique was used to determine genetic diversity of *C. albicans* isolates. The criteria for genotyping were the differences in banding positions of each isolate in the RAPD-PCR profile. The isolates with identical banding profiles were pooled into a single genotype. Similarity coefficient ≥ 80% was the value that was arbitrarily used as the threshold for clustering of similar genotypes, since it is roughly halfway between the mean value for dissimilarity and identity. On the basis of RAPD-PCR profiles, among 52 strains of *C. albicans*, 34 genotypes were defined within the overall yeast population, including 10 clusters containing from 2 to 6 isolates, which comprised 28 (53.85%) strains and unique genotypes represented 24 (46.15%) strains. The coefficient of relatedness of all isolates was 17% (Fig. 1).

**DISCUSSION**

Genotyping by RAPD-PCR technique is easy to perform, versatile, useful and a reliable method allowing for genetic and phylogenetic differentiation of strains belonging to *Candida* spp., including *C. albicans*, and may be applied as a valuable method for epidemiological purposes (Chong et al., 2003; Samaranayake et al., 2003; Samaranayake et al., 2011). According to data in the literature (Pinto et al., 2004; Noumi et al., 2009; Biernasiuk et al., 2010; Gültekin et al., 2011; Samaranayake et al., 2011), RAPD-PCR is a good method for analyzing the *C. albicans* genotype, greatly dependent on the choice of primers. Several primers could be used for

![Figure 1. Dendrogram generated for 52 isolates of *C. albicans* from upper respiratory tract of patients with lung cancer on the basis of RAPD-PCR profiles.](image-url)
REFERENCES


