Stability of Karyotype in Serial Isolates of *Candida albicans* from Neutropenic Patients

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Serial isolates of *Candida albicans* were obtained from 20 patients with leukemia over periods of up to 8 months. The fingerprinting of these isolates by interrepeat PCR and random amplified polymorphic DNA PCR has been described previously (A. van Belkum, W. Melchers, B. E. de Pauw, S. Scherer, W. Quint, and J. F. Meis, J. Infect. Dis. 169:1062–1070, 1994). Contour-clamped homogeneous electric field gel electrophoresis was used to examine the chromosomes of these isolates. When changes in the karyotype were seen in a series of isolates, additional interrepeat PCR and Southern blotting with a repeat DNA sequence from the 27A family were performed. These two genotyping tools were used to determine if karyotypic changes seen in a series of isolates were due to chromosome rearrangements in a single strain or due to colonization with more than one strain. It was determined that changes in karyotype in a series of strains indicated infection by a new strain.

In recent years it has become obvious that in the important opportunistic pathogen *Candida albicans*, there is a high degree of strain variability. Certain strains are able to switch between different forms varying in colony and cellular morphology, karyotype, antigenicity, resistance to antifungal agents, and other traits at frequencies as high as 10^{-1} to 10^{-2} (12). Chromosomal rearrangements are often, though not invariably, associated with this phenotypic variation (3). This association suggests that in phenotypically unstable strains there is an underlying genomic instability. This genetic variability can be seen as karyotypic instability in certain strains of *C. albicans* during passage in vitro (3). However, the importance of genetic and phenotypic variation to this organism in vivo, during episodes of infection, is not known.

Limited studies of chromosomal variation in *Candida* species during an infection have been made. Asakura et al. (1) compared pairs of isolates of *C. albicans* and *Candida (Torulopsis) glabrata* isolated 1 or more months apart from several candida vaginitis patients and found changes only in the rDNA gene (rDNA)-bearing chromosome. More recently, in a survey of five AIDS patients, karyotypes of serial *C. albicans* isolates showed no changes apart from variation in chromosomes hybridizing with rDNA (2). The chromosome with the rDNA repeats, termed chromosome R in the numbering system of Scherer and Magee (9), has been observed to vary considerably in size in almost all strains of *C. albicans* that have been studied and also in other organisms. This variation in size is thought to be due to unequal crossing-over between the rDNA repeats on the two homologs and is assumed to represent a background level of recombination.

Van Belkum et al. (13) recently typed sequentially collected isolates of *C. albicans* from 25 patients with leukemia using interrepeat PCR. They used a variety of primers from both prokaryotic and eukaryotic sources. It was found that while for most patients consecutive isolates of *C. albicans* exhibited identical PCR genotypes, for some patients isolates showed

occasional genotypic changes. These changes represented either colonization by a new strain or genotypic change of an existing one. Whilst highlighting the importance of PCR as a typing tool, these results suggested the presence of a low level of genomic instability in some *C. albicans* strains during an infection. We present here the karyotyping results for the *C. albicans* strains used in that study, together with results of Southern blotting with the 27A repeat sequence. Additional interrepeat PCR was performed to rule out mixing of strains. We demonstrate the surprising chromosomal stability of all of the isolates from these patients.

MATERIALS AND METHODS

Serial isolates of *C. albicans* from 21 neutropenic patients at University Hospital Nijmegen, Nijmegen, The Netherlands, were maintained on yeast extract-peptone-glucose (YPD) agar plates and grown in YPD broth as previously described (11). The identification of these isolates as *C. albicans* was originally by germ tube and chlamydospore formation (13) and was confirmed from the basic pattern of the karyotype (number and approximate size of chromosomes). Samples of DNA for contour-clamped homogeneous electric field (CHEF) gel electrophoresis were prepared as described by Phillipsen et al. (7). CHEF gels were prepared and run as previously described (3). Southern blotting analysis of restriction digests and selected CHEF gels was as described by Barton and Scherer (3). A plasmid subclone, clone 824, from the 27A family (10) was used as a probe for Southern analysis. Interrepeat PCR was performed as described previously with primers 1245 and 1246 (ERIC2 and ERIC1, respectively [13]).

RESULTS

Inspection of the CHEF gels of the 21 series of isolates obtained from the 21 patients showed two classes of chromosome variation. In almost all series there was slight variation in the relative migration of one of the largest chromosomes. This variation in chromosome R has been observed before by us and other workers and occurs in vitro and in vivo (1, 5). Apart from this variation the karyotypes of most series of strains were remarkably consistent, while the karyotypes of the different series differed slightly, e.g., strains from patients I and T had different karyotypes (Fig. 1). A similar consistency of karyotypes in other collections of serially isolated *C. albicans* strains has been noted (data not shown).

However, a second class of chromosome change was seen in four different series in which more than one karyotype was

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FIG. 1. Karyotypes of serial isolates of *C. albicans* from two patients (patients I and T). Note the appearance of a homolog of chromosome R in the last two isolates from patient I (see arrow). Each lane contains a different strain in the order isolated.

observed (Table 1; Fig. 2). Differences ranged from the appearance of a single extra band to multiple changes in the relative migration of chromosome bands. Strains from patients J and M both consisted of two identical karyotypes and one that differed by a single band (Fig. 2). In strains from patients D and P there were isolates with greater differences between karyotypes, involving more than one chromosome (Fig. 2), as well as a more subtle variant in patient D (isolate 6611). In patient P, two isolates taken on the same date from different locations (oral and fecal isolates) possessed identical karyo-

types and were replaced at a later date by two isolates from the same sites with a different karyotype.

To determine if these variant karyotypes represented the same strain that had undergone a chromosomal rearrangement or were different strains, DNAs from these series were compared by 27A fingerprinting following digestion with *Eco*RI. In addition, the interrepeat PCR typing method of van Belkum et al. (13) was used. The results are shown in Fig. 2 and Table 1. In all cases, when a variant karyotype arose in a series of clinical isolates of *C. albicans*, PCR and Southern blotting with the 27A sequence indicated that there were changes at a large number of sites throughout the genome. There was good agreement between the three typing methods. This observation supports the use of karyotyping alone to define strains collected in two recent epidemiological studies (6, 14).

DISCUSSION

By using additional typing methods, this study demonstrates that a change in the karyotype in serial clinical isolates of *C. albicans* represents colonization by a new strain.

The possibility remains that the karyotypic variants represent strains that have undergone profound genomic changes affecting both karyotype and genotype. However, initial work with the 27A sequence showed that the fingerprint pattern of strains is very stable despite in vitro passaging and changes in drug resistance (10). Further work suggested that morphological and karyotypic changes can occur with only minor changes in the genotype, as judged by the 27A fingerprint (4). The latter result is to be expected, since the large-scale recombinations that generate visible chromosomal change are unlikely to grossly affect fragments with repeated sequences that are assayed by PCR or 27A fingerprinting.

This study also demonstrates the good agreement between three genotyping methods. Minor differences between closely related strains with identical karyotypes revealed by PCR and 27A fingerprinting represent background variation and confirm the greater sensitivity of those two typing methods. One

TABLE 1. Typing of serial *C. albicans* isolates from neutropenic patients^a

Patient	Isolate no.	Type or source of isolate ^b	Isolation date (mo/day/yr)	Karyotype	PCR genotype ^c	27A fingerprint ^c
D	5909	f	12/27/91	D1	D1	D1
	5910	ms or ts	12/27/91	D2	D2	D2
	6009	ms or ts	01/29/92	D1	D1	D1
	6060	ms or ts	02/20/92	D1	D1	D1
	6073	f	02/27/92	D1	D1	D1
	6424	ms or ts	06/22/92	D1	D1	D1
	6611	f	07/29/92	D3	D3	D3
J	6697	ms or ts	08/13/92	J1	J1	J1
	6711	f	08/17/92	J2	J2	J2
	6735	ms or ts	08/24/92	J2	J2	J2
M	5418	ms or ts	08/08/91	M1	M1	M1
	5520	ms or ts	09/02/91	M1	M1	M1
	5705	f	10/22/91	M2	M2	M2
P	4289	f	08/08/90	P1	P1	P1
	4290	ms or ts	08/08/90	P1	P1	P1
	4583	ms or ts	11/05/90	P2	P2	P2
	4584	f	11/05/90	P2	P2	P2

^a Karyotype, PCR genotype, and 27A fingerprint designations consist of the letter used to identify the patient and a number. Different numbers indicate completely different types.

^b f, fecal isolate; ms, mouth swab; ts, throat swab.

^c Minor differences are not indicated but can be seen in Fig. 2.

FIG. 2. Karyotypes of serial isolates of *C. albicans* from four patients (patients D, J, M, and P) (panels A), together with fingerprints obtained with a probe from the 27A repeat family (panels B) and the interrepeat PCR type (panels C). For a list of strain numbers, see Table 1. Each lane contains a different isolate.

advantage of karyotyping and fingerprinting with a species-specific probe such as the 27A sequence is that, unlike the situation with many PCR typing methods, species identity can be confirmed.

Thus, the karyotypes of strains infecting these neutropenic patients are stable over a period of up to 8 months. Colonization by new strains is the primary source of karyotypic variation. This result is perhaps surprising, since extensive karyotypic variability in vitro in some strains has been described (3). The fact that most strains of *C. albicans* have distinctive karyotypes indicates that chromosome rearrangements must be occurring over time. However, it is clear that this kind of genomic instability occurs less frequently in vivo than previously thought (8). Little is known about how the host environment affects genetic and/or phenotypic variation. When the pathogen is not under pressure from the immune system, as in neutropenic patients, there may be a lower level of chromosome and perhaps other variation. This hypothesis is currently under investigation in our laboratories.

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REFERENCES

- Asakura, K., S.-I. Iwaguchi, M. Homma, T. Sukai, K. Higashida, and K. Tanaka. 1991. Electrophoretic karyotypes of clinically isolated yeasts of *Candida albicans* and *C. glabrata*. *J. Gen. Microbiol.* **137**:2531-2538.
- Bart-Delabesse, E., P. Boiron, A. Carlotti, and B. Dupont. 1993. *Candida albicans* genotyping in studies with patients with AIDS developing resistance to fluconazole. *J. Clin. Microbiol.* **31**:2933-2937.
- Barton, R. C., and S. Scherer. 1994. Induced chromosome rearrangements and morphologic variation in *Candida albicans*. *J. Bacteriol.* **176**:756-763.
- Barton, R. C., and S. Scherer. Unpublished data.
- Barton, R. C., D. Schmidt, and S. Scherer. 1994. Analysis of karyotype in *Candida albicans*, p. 99-104. In B. Maresca and G. S. Kobayashi (ed.), *Molecular biology of pathogenic fungi: a laboratory manual*. Telos Press, New York.
- Pfaller, M. A., J. Rhine-Chalberg, S. W. Redding, J. Smith, G. Farinacci, A. W. Fothergill, and M. G. Rinaldi. 1994. Variations in fluconazole susceptibility and electrophoretic karyotype among oral isolates of *Candida albicans* from patients with AIDS and oral candidiasis. *J. Clin. Microbiol.* **32**: 59-64.
- Phillipsen, P., A. Stotz, and C. Scherf. 1991. DNA of *Saccharomyces cerevisiae*. *Methods Enzymol.* **194**:169-182.
- Rustchenko-Bulgac, E. P., F. Sherman, and J. B. Hicks. 1990. Chromosomal rearrangements associated with morphological mutants provide a means for genetic variation of *Candida albicans*. *J. Bacteriol.* **172**:1276-1283.
- Scherer, S., and P. T. Magee. 1990. Genetics of *Candida albicans*. *Microbiol. Rev.* **54**:226-241.
- Scherer, S., and D. A. Stevens. 1988. A *Candida albicans* dispersed, repeated gene family and its epidemiologic applications. *Proc. Natl. Acad. Sci. USA* **85**:1452-1456.
- Sherman, F., G. R. Fink, and C. W. Lawrence. 1978. *Methods in yeast genetics*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Soll, D. R. 1992. High-frequency switching in *Candida albicans*. *Clin. Microbiol. Rev.* **5**:183-203.
- Van Belkum, A., W. Melchers, B. E. de Pauw, S. Scherer, W. Quint, and J. F. Meis. 1994. Genotypic characterization of sequential *Candida albicans* isolates from fluconazole-treated neutropenic patients. *J. Infect. Dis.* **169**:1062-1070.
- Voss, A., R. J. Hollis, M. A. Pfaller, R. P. Wenzel, and B. N. Doebbeling. 1994. Investigation of the sequence of colonization and candidemia in non-neutropenic patients. *J. Clin. Microbiol.* **32**:975-980.