Expression of CDR1, a multidrug resistance gene of Candida albicans: transcriptional activation by heat shock, drugs and human steroid hormones

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Received 4 November 1997; revised 5 January 1998; accepted 10 January 1998

Abstract

We have examined the expression of CDR1 (Candida drug resistance gene) in different stress conditions. There was a significant but transient enhancement of CDR1 expression associated with elevated temperatures. Most noteworthy transcriptional activation was observed with miconazole and vinblastine. Interestingly, β-estradiol and progesterone were also able to enhance CDR1 expression. Elevated levels of CDR1 and CDR2 (a homologue of CDR1) mRNA were found in some azole-resistant clinical isolates of C. albicans. CaMDR1 (benomyl-resistant) expression, however, did not differ among all the resistant isolates. Our results confirm the existence of multiple mechanisms of azole resistance in C. albicans.

Keywords: ABC protein; Multidrug resistance; Expression; Stress induced; Candida albicans

1. Introduction

The dimorphic pathogenic yeast Candida albicans is naturally more resistant to several drugs, e.g. cycloheximide, benomyl and methotrexate, than Saccharomyces cerevisiae [1,2]. In addition, the incidence of C. albicans cells acquiring resistance to azoles and polyenes has increased considerably in recent years, which has posed serious problems for its successful chemotherapy [3]. Azole resistance in C. albicans can arise by several mechanisms, viz., mutations in the target enzyme, P450DM [4] or lesions in other ergosterol biosynthetic enzymes (e.g. Δ5,6 sterol desaturase) [5], which compensates for the inhibition of P450DM by azoles. In addition, energy-dependent drug transporters of the multidrug resistance (MDR) type have been implicated in azole resistance [2,4]. Three C. albicans proteins, encoded by the CDR1, CDR2 and CaMDR1 (benomyl-resistant) genes, have been shown to play a role in fluconazole resistance [4,6–8].

In an attempt to delineate the functional role of CDR1, we have investigated factors which influence
CDRI expression. We demonstrate that CDRI expression is induced by environmental stimuli, e.g. temperature, drugs and human steroid hormones. The expression also appears to be growth stage-specific. The expression pattern of CDRI, CDR2 and CaMDR1 in azole-resistant clinical isolates of C. albicans confirms the existence of multiple mechanisms for azole resistance in C. albicans.

2. Materials and methods

2.1. Yeast isolates and culture conditions

C. albicans ATCC 10261 and all other clinical isolates used in this study were routinely grown and maintained in YEPD medium (yeast extract 1%, bacteropeptone 2%, glucose 2%) at 30°C. Clinical isolates were a kind gift from Tanya Parkinson and Chris Hitchcock, Pfizer Ltd. Fluconazole was kindly provided by Pfizer Ltd., Sandwich, Kent, UK. Itraconazole and ketoconazole were kind gifts from the Janssen Research Foundation, Beerse, Belgium. All the chemicals including steroids and other drugs were from Sigma, and were of analytical grade.

2.2. Isolation of total RNA and Northern analysis

Total RNA isolation from C. albicans cells was done as described earlier [9]. For Northern analysis the standard protocol was used [10]. Northern transfer was performed overnight as given in the standard laboratory protocol [10] using Hybond N\textsuperscript{+} nylon membrane (Amersham). RNA was fixed by UV cross-linking (Stratagene) as per the manufacturer’s instructions. A 4.2-kb BamHI fragment of CDRI or CDR2 (PCR probe) or CaMDR1 (1.8-kb EcoRV fragment) as described by Sanglard et al. [7], labelled with [\(\alpha^{32}\text{P}\)]dCTP using the random primer kit from Life Technologies (USA), was used as molecular probe for Northern analysis [6].

3. Results

3.1. CDRI expression is temporally regulated

In order to ascertain the physiological role of CDRI as well as its role in drug resistance, CDRI mRNA levels were checked during the growth phase (Fig. 1a). The level of expression of CDRI was higher at 4 h and 6 h of growth (lanes 1 and 2, Fig. 1b),
declined during mid-exponential phase (lanes 4 and 5, Fig. 1b) and again increased at late exponential and stationary phase (lanes 6 and 7, Fig. 1b). Normalised densitometric scans in Fig. 1b showing statistical variations also confirm that CDRI transcription is temporally regulated (Fig. 1c). It must be pointed out that the both 8-h and 10-h points (Fig. 1c) do not depict any bars since there were no significant variations observed at these hours of growth.

3.2. Heat shock and unrelated drugs induce expression of CDRI

The sequencing of CDRI has earlier revealed the existence of two putative heat shock elements (HSE) at −192 and −323 bp upstream of the translation start site [6]. In order to study if heat stress could induce expression of CDRI, C. albicans cells were grown at 30°C up to 10 h, were transferred to 37°C or 42°C and were harvested after 15, 30 and
60 min of incubation (lanes 2–7, Fig. 2a). As a control, an aliquot of cells was incubated at 30°C for the same period of time. There was a significant increase in expression of CDR1 after 15 min of exposure of cells to 37°C or 42°C, after which transcript levels decreased gradually (Figs. 2a and 3a).

CDR1 was previously shown to confer resistance to several drugs and metabolic inhibitors [6]. We therefore examined whether some of these compounds could induce transcription of CDR1. Cycloheximide caused an increase in CDR1 mRNA levels (Figs. 2b and 3b), which was time-dependent and of longer duration than the temperature-induced expression. Maximal induction was obtained using 0.25 μg ml⁻¹ cycloheximide and addition of higher concentrations did not result in any further increase in mRNA levels (lanes 2–4, Figs. 2b and 3b).

A range of drugs was tested for their effects on CDR1 transcription, including antifungal agents (miconazole, fluconazole and nystatin), a transcription inhibitor (o-phenanthroline) and anticancer drugs (verapamil and vinblastine). All of the drugs tested caused an increase in CDR1 mRNA levels after 60 min exposure, with miconazole having the greatest effect (lane 3, Figs. 2c and 3c).
3.3. CDR1 expression is induced by L-estradiol and progesterone

In this study, we have for the first time demonstrated that human steroid hormones can transcriptionally activate CDR1 promoter. The CDR1 transcript levels were monitored in the presence of L-estradiol and progesterone (Fig. 2d). The steroid-induced activation was very rapid which was evident within 15 min of exposure of cells to the hormones (Fig. 2d). L-Estradiol and progesterone were both able to enhance CDR1 transcript levels to about 4.5-fold as compared to the control (Figs. 2d and 3d) within 15–30 min of exposure.

3.4. Some of the resistant clinical isolates of C. albicans have overexpressed CDR1 and CDR2

We have examined CDR1 mRNA levels in fluconazole-resistant clinical isolates (MICs 50–100 μg ml⁻¹). It is evident from Fig. 4 that isolates Y01.547 and Y01.549 (lanes 2 and 3) particularly showed high expression of CDR1 as compared to other strains. Interestingly, isolates Y01.553 and Y01.584, which did not show high levels of CDR1 transcript, exhibited enhanced levels of expression of CDR2. On the other hand, CaMDR1 expression did not vary significantly in these isolates. It must be mentioned that a low level of expression of all three genes, viz. CDR1, CDR2 and CaMDR1, was observed in four azole-sensitive isolates of C. albicans (data not shown).

4. Discussion

The human multidrug resistance MDR1 gene promoter has been shown to contain heat shock consensus elements and is induced in response to elevated temperatures and to other chemical stress-inducing agents [11]. PDR5 (YDR1/STS1) and SNQ2, the S. cerevisiae homologues of CDR1, have been shown to be stimulated by stress [12,13]. Since the CDR1 gene contains two heat shock (stress) elements at positions -192 and -323 bp upstream of the translation start site [6], we analysed CDR1 expression under different stress conditions, including high temperature and drug treatment, and showed that CDR1 is also a stress-inducible gene. CDR1 mRNA levels also increased on entry into stationary phase, in common with many other genes which are known to be stress-induced [14]. Several unrelated drugs were also able to induce CDR1 transcription. The overexpression induced by miconazole, nystatin and vinblastine was most noteworthy.

If one considers the fact that an increase in sex hormone levels leads to a rise in the incidence of Candida infections, our present results are significant. It is tempting to speculate that the hormonal environment of the host may affect drug resistance of C. albicans. In this regard it is pertinent to mention a recent report where it has been shown that in S. cerevisiae, the human steroid hormone β-estradiol is transported through Pdr5p and Snq2p and the disruption of their genes resulted in the accumulation of β-estradiol [15,16]. Our earlier results also indicate that β-estradiol and corticosterone are substrates of Cdr1p [17]. The presence of corticosteroid and estrogen binding proteins in C. albicans and other species
of Candida has also been established [18]. In the light of these results, our study suggests a possibility of a steroid-receptor cascade linked to multidrug resistance of C. albicans. The fact that human steroid responsive element (SRE) is present in the promoter region of CDR1 (Prasad et al., personal communication) supports our argument. Whether the transient increase in CDR1 expression, induced by drugs and steroid hormones in this study, has any clinical relevance or only represents a general stress response to xenobiotic exposure in growth cultures remains to be seen.

We have shown that some of theazole-resistant isolates of C. albicans show high levels of expression of CDR1 and of CDR2, particularly in those isolates where CDR1 expression was low (Fig. 4). The expression of CaMDR1 did not vary significantly in these isolates. In conclusion, we have demonstrated in this study that CDR1 expression is growth phase-specific and is inducible by short exposure of cells to elevated temperatures, unrelated drugs and human steroid hormones. The difference in the levels of CDR1, CDR2 and CaMDR1 expression in theazole-resistant isolates suggests multiple mechanisms of resistance. Functional analyses of other multidrug resistance genes (Prasad et al., unpublished results) would certainly clarify the mechanisms of drug resistance in C. albicans.

Acknowledgments

We would like to thank Tanya Parkinson and Chris Hitchcock, Pfizer, UK for providing clinical isolates of C. albicans. We are grateful to Kailash Upadhyaya and S.E. Hasnain for their critical advice and to Pranab Mukherjee for his help in finalising the manuscript. Our sincere thanks to Shehnaz and Sher Ali for densitometric analysis of autoradiograms and to R.N. Saini for photographic assistance. The work presented in this paper has been supported by a grant to one of us (R.P.) from the Department of Biotechnology (BT/R and D/15/02/94), India. S.K.M., V.G. and S.P. acknowledge the fellowships awarded by the University Grants Commission.

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