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Research Article

Metalloproteinases Secreted by Candida: A Systematic Review

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ABSTRACT

The aim of this article was to identify if the metalloproteinases secreted by Candida would be considered a factor of patogenicity, through a systematic review of literature. The eletronic search were conducted in three databases: MEDLINE via PubMeb, Scopus and Web of Science. The inclusion criteria was only in vitro assays concerning to metalloproteinases secreted by Candida. After the database search [Pubmed (94), Scopus (9) and Web of Science (8)] and removal of duplicates, 100 studies were identified. After title and abstract reading screening, 11 studies remained, and this number was reduced to nine after full-text reading screening. Candida metalloproteinases may play a role in the degradation of the subendothelial extracellular matrix components and facilitate the migration of the yeast in the tissues after crossing the endothelial layer, allowing the fungal invasion of target organs. Thus, the inhibition of metalloproteinase may have therapeutic implications for controlling pathological collagen breakdown. This represents a promising approach to the treatment of infectious diseases. Metalloproteinases secreted by Candida is one factor of pathogenicity of this fungi genus and represents a promising approach to the treatment of candidiasis. However, research in this field needs advancement and improvement in terms of rigor and quality of the studies involved with this theme.

Keywords: Candidiasis; Candida spp; metalloprotease; systematic review.

INTRODUCTION

The incidence of fungal infections in humans has increased considerably over the decades. It may be attributed to the rise of the AIDS epidemic, the increasing number of patients at risk, such as those with advanced age and those who have undergone major surgery, immunosuppressive therapy, and solid-organ and hematopoietic stem cell transplantation, among others [1,2,3]. In addition, despite their wide use, antifungal agents have certain limitations due to their side effects, such as toxicity and the emergence of resistant strains, especially those resistant to fluconazole via the efflux pump mechanism [4,5].

Of the fungi regarded as human pathogens, the members of the genus Candida are the most frequently recovered from human fungal infection. Of the Candida species isolated from humans, C. albicans is the most prevalent under both healthy and disease conditions; however, non-C. albicans species such as C. glabrata, C. tropicalis and C. parapsilosis can be frequently identified as human pathogens [6,7].

Candida pathogenicity is facilitated by a number of virulence factors, most importantly adherence to host surfaces including medical devices, biofilm formation and secretion of hydrolytic enzymes. These enzymes can facilitate the spread of this yeast to deeper organs, as well as protect it from the humoral immune response of the host [8,9].

The term protease is synonymous with peptidase, proteolytic enzyme and peptide hydrolase. The proteases include all enzymes that catalyse the cleavage of the peptide bonds (CO-NH) of proteins, digesting these proteins into peptides or free amino acids. The proteases are initially classified following their mode of action and their active sites: Aspartic, cystein, metallo, serine, and threonine proteases [10,11].

Candida albicans expresses a vast number of hydrolytic enzymes, playing roles in several phases of yeast–host interactions [12]. Candida spp. metalloproteinase may represent a new pathogenic factor for degrading components of the extracellular matrix of the host and the inhibition of metalloproteinase may have therapeutic implications since it can control the pathological breakdown of collagen, which represents a promising approach for the infectious diseases [8,9,13].

Although it is now clear that proteases may act differently as virulence factors, knowledge on protease substrate specificities remains rather poor and few studies have focused on the research of specific inhibitors [14].

The virulence factors and mechanisms of invasion of Candida spp. are the subject of many research groups; however, many of their interactions with the host are singularly complex and still remains

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poorly understood. Following focused question, "Metalloproteinases secreted by Candida: is there some evidence in the published literature to identify if this metalloproteinase would be considered a factor of patogenicity", through a systematic review of literature.

METHODS

Information sources and search

To identify studies to be included for this review, we searched on the electronic databases MEDLINE via PubMeb, Scopus and Web of Science. No restrictions were placed on the publication date or languages. The search strategies defined for the databases described above are listed below (Table 1). The search strategy was appropriately modified for each database and performed by two reviewers (J.S. and R.L.) to identify eligible studies. Full-text versions of the studies that appeared to meet the inclusion criteria were retrieved for further assessment and data extraction.

Table1: Database screening [PubMed (94), Scopus (9) and Web of Science (8)].

Search strategy
Pubmed
("metalloproteases"[MeSH Terms] OR "metalloproteases"[All Fields] OR "metalloproteinases"[All Fields]) AND ("candida"[MeSH Terms] OR "candida"[All Fields])
Scopus
TITLE-ABS-KEY ("metalloproteases") AND TITLE-ABS-KEY ("candida")
Web of science
TS=("metalloproteases" AND "candida")

Inclusion criteria for included studies

All studies that describe the metalloproteinases secreted by Candida spp. were included.

RESULTS

Study selection

After the database searching [Pubmed (94), Scopus (9) and Web of Science (8)] and removal of duplicates, 100 studies were identified, according to Figure 1. After title and abstract reading screening, 11 studies remained, and this number was reduced to nine after the full-text reading screening, meeting the inclusion criteria: studies at analyzed the metalloproteinase expression by Candida.

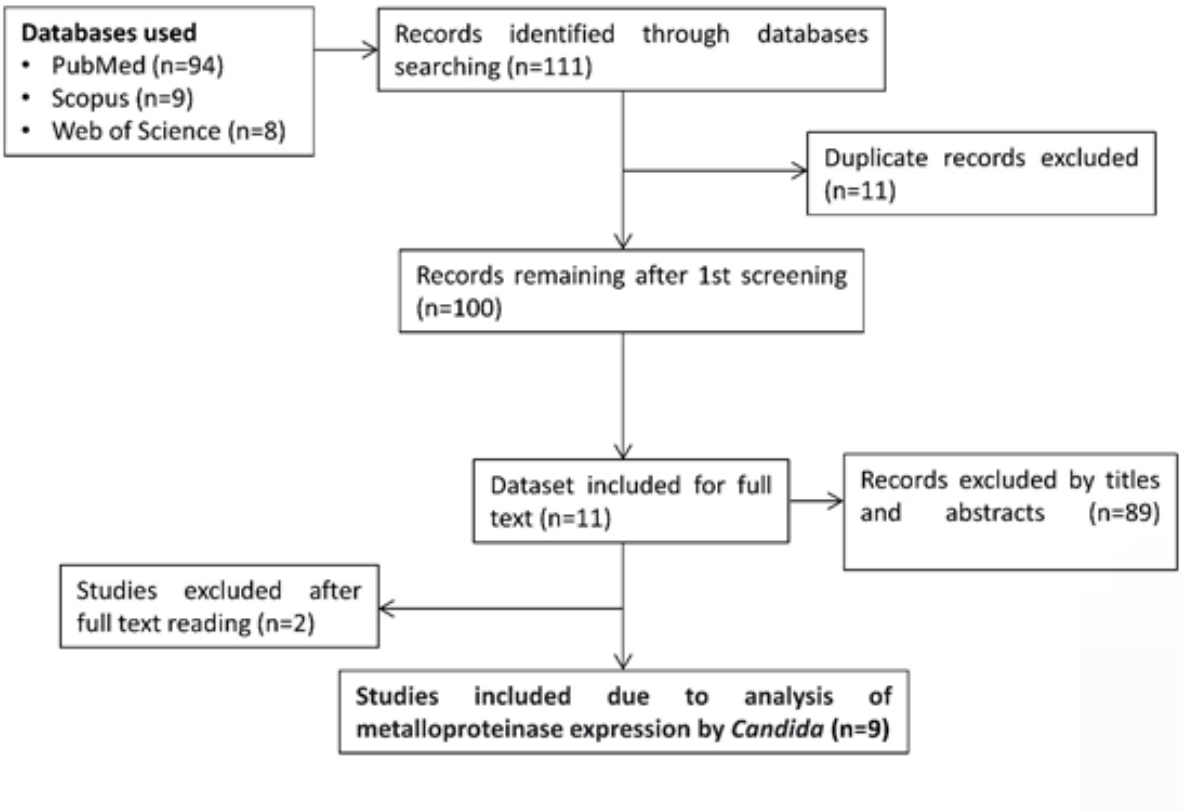

Figure 1: Flow diagram of the study

Characteristics of included articles

The characteristics of included articles are show in the Table 2. Four studies detected and purified a metalloproteinase from C. albicans [15,16,17,18], one study used this same metalloproteinase for the development of an immunoassay enzyme [19], two works investigated the ability of this purified metalloproteinase to degrade proteins of the extracellular matrix [20,21] and two studies investigated the influence of the agents antifungals for inhibition of the secretion of metalloproteinase purified from C. albicans [22,23].

Table 2: Characteristics of the studies included.

Study	Assay	Material evaluated	Results
El Moudni et al., 1995 [15]	Purification and characterisation of a metallopeptidase of Candida albicans by high performance liquid chromatography.	C. albicans strain ATCC 2091 and nine clinical Candida isolates were used: three C. albicans isolates from urine, vagina and the mouth, and single isolates of C. tropicalis, C. glabrata, C. parapsilosis, C. krusei, C. yuilliermondi and C. pseudotropicalis.	A novel aminopeptidase was purified from an extract of C. albicans. The enzyme was strongly inhibited by specific metallo-enzyme inhibitors-EDTA and o-phenanthroline. There is evidence that a similar or identical enzyme occurs in other C. albicans clinical isolates and other Candida spp.
El Moudni et al., 1998 [19]	Development of an enzyme-linked immunosorbent assay to detect antibodies directed against this antigen in sera from patients with candidiasis.	A Candida albicans 52-Kilodalton Metallopeptidase from C. albicans strain ATCC 2091.	Diagnostic parameters show high diagnostic specificity of 97% and a sensitivity of 83% at a cutoff value of 0.425 and suggest the usefulness of this aminopeptidase for the diagnosis of systemic candidiasis.
Rodier et al., 1999 [20]	Studied the degradation of four constitutive proteins of the extracellular matrix: type I and IV collagens, laminin and fibronectin, by a 95-kDa metallopeptidase, localised in the cell wall of C. albicans. Each of these constituents was incubated with the purified enzyme and its degradation products analysed by a lectrophoretic method.	95-Kda metallopeptidase, localised in the cell wall of c. *Albicans*	Observed that type I collagen and fibronectin were totally degraded by the enzyme whereas type IV collagen and laminin were only partially degraded. The C. albicans metallopeptidase may play a role in the degradation of the subendothelial extracellular matrix components. This enzyme could facilitate the migration of the yeast in the tissues after crossing the endothelial layer, allowing the fungal invasion of target organs.
Imbert et al., 2002 [22]	Tested six compounds on the C. albicans enzyme. Doxycycline, gentamicin, cefalothin, galardin, and elaidic and oleic acids are known for their capacity to inhibit some inhibitors of matrix metalloproteases.	*C. albicans enzyme.*	Only oleic acid was able to markedly inhibit the purified metallopeptidase at very low concentrations. Moreover, this fatty acid inhibited the secretion of the enzyme in the culture medium without altering the yeast viability.
de Brito Costa et al., 2003 [16]	In the present study, gelatin-SDS PAGE analysis was used to characterize extracellular proteinases in 44 oral clinical isolates of C. albicans.	Oral clinical isolates of C. albicans from HIV-positive and healthy children.	Our survey indicates that these oral clinical isolates of C. albicans have complex extracellular proteolytic activity profiles, which illustrates the heterogeneity of this species. We showed four distinct proteolytic patterns composed of distinct serine (30^58-kDa) and metalloproteinase (64^95-kDa) activities.

dos Santos et al., 2006 [17]	Detect metallopeptidades using gelatin–sodium dodecyl sulfate polyacrylamide gel electrophoresis and establish a probable functional implication for these novel peptidase classes.	*C. albicans isolated from the infected urine.*	This study showed for the first time the capability of an extracellular proteolytic enzyme other than aspartic-type peptidases to cleave a broad spectrum of relevant host proteinaceous substrates by the human pathogen C. albicans.
Imbert et al., 2006 [23]	Effect of azoles on the secretion of a C. albicans metallopeptidase.	Eight isolates C. albicans have been cultivated in presence of MIC, MIC/2 and MIC/4 of voriconazole, fluconazole and itraconazole.	Demonstrate in this study that the in vitro secretion of a metallopeptidase could be modified during the growth of C. albicans with subinhibitory concentrations of some azoles.
Klinke et al., 2008 [21]	The proteolytic potential of the pathogenic fungus C. albicans was evaluated by the identification and functional characterization of a peptidolytic enzyme isolated from the cell wall of the microorganism.	*C. albicans strain ATCC 2091.*	The present study identify and characterize the enzyme CaApe2 as a neutral metalloaminopeptidase of still unknown function that, due to secretion, can be isolated from the cell wall of the pathogenic yeast C. albicans.
Yavuz et al., 2017 [18]	Production, purification, and characterization of a thermostable metalloprotease from an environmental strain of candida kefyr 41 psb	*C. Kefyr strain 41 psb.*	Characterization of a thermostable metalloprotease from C. kefyr 4 PSB, determining the temperature of its maximum activity and its stability in the presence of organic solvents.

DISCUSSION

Studies involving Candida metalloproteinase are poorly described in the literature. Some metalloproteinases have been purified and characterized, but little is known about the function of these enzymes as potential pathogens.

The first study about metalloproteinase from Candida albicans was by El Moudni et al. [15] that purified a metalloproteinase from C. albicans by high performance liquid chromatography (HPLC). This enzyme was originally described as a protein of 52 kDa with optimal activity at pH 7.2. The same authors in 1998 [19] used this same metalloproteinase for the development of an immunoassay enzyme (ELISA) to detect antibodies directed against this antigen in the serum of patients with candidiasis. The study by El Moudni et al. [19], used the purified metalloproteinase and showed satisfactory values for diagnostic specificity (97.15%) and sensitivity (83%), demonstrating clearly that the use of a purified antigen of C. albicans, instead a crude extract, provides a more specific test for the diagnosis of systemic candidiasis.

According to Rodier et al. [20] other proteolytic enzymes could play a role in the degradation of extracellular matrix proteins. In his work they purified a metalloproteinase of C. albicans, located in the cell wall, and reported that this enzyme, previously described as a 52 kDa protein was indeed recovered in a native form of 95 kDa. Thus, they investigated the ability of this 95kDa metalloproteinase to degrade four proteins of the extracellular matrix (collagen type I and IV, laminin and fibronectin). They incubated the purified enzyme with each of these components analyzed its degradation products by an electrophoresis method. It was observed that the enzyme totally degraded collagen type I and fibronectin, but only partially degraded laminin and type IV collagen. These findings showed that C. albicans metalloproteinase may play a role in the breakdown of subendothelial extracellular matrix components of the host and promotes fungus migration after crossing the endothelial layer. And it allows the invasion of the fungus of the target organ and, therefore, can be considered a factor pathogenicity of C. albicans [20].

Inhibition of metalloproteinase may have therapeutic implications, since it can control the pathological collagen degradation, thus representing a promising approach for the treatment infectious diseases.

A study conducted by Imbert et al. [22] tested the inhibitory effect of doxycycline, cephalothin, gentamicin, galardin, and oleic and elaidic acids on C. albicans 95 kDa metalloproteinase in relation to the enzyme's ability to degrade components of the extracellular matrix. Among these agents, only the oleic acid was able, significantly, to inhibit purified metalloproteinase in low concentrations. Oleic acid acted not only on the enzyme directly, but also on its secretion into the culture medium in a dose-dependent way without altering the viability of the yeast.

Later in 2006, the same researchers investigated the influence of three azole antifungals on the secretion of 95kDa metalloproteinase purified from C. albicans. Eight isolates were grown in the presence of voriconazole, fluconazole and itraconazole in MIC, MIC/2 and MIC/4. Voriconazole and fluconazole decreased the secretion of metalloproteinase. However, itraconazole increased the secretion of the enzyme in three isolates [23].

A study by de Brito Costa et al. [16], with the purpose of characterizing the proteinases secreted in oral, clinical isolates of C. albicans showed a profile of extracellular proteolytic activity rather complex and boosted the heterogeneity of this species. In this study, four distinct patterns of extracellular proteolytic activity of serines (30-58 kDa) and metalloproteinases (64-95 kDa) were detected, demonstrating that the number of proteinase enzymes produced

ranged between strains of the same species. The variability in expression or activity of proteinases may be partly responsible for the immune response after the initial infection.

In addition, dos Santos et al. identified two novel extracellular peptidase classes in C. albicans. Using gelatin–sodium dodecyl sulfate polyacrylamide gel electrophoresis two gelatinolytic activities were detected at physiological pH: a 60 kDa metallopeptidase, completely blocked by 1,10-phenanthroline, and a 50 kDa serine peptidase inhibited by phenylmethylsulfonyl fluoride. In an effort to establish a probable functional implication for these novel peptidase classes, they demonstrated that the 50 kDa secretory serine peptidase was active over a broad pH range (5.0-7.2) and was capable to hydrolyze some soluble human serum proteins and extracellular matrix components. Conversely, when this isolate was grown in yeast carbon base supplemented with bovine serum albumin, a secretory aspartyl peptidase activity was measured, instead of metallo and serine peptidases, suggesting that distinct medium composition induces different expression of released peptidases in C. albicans [17].

Kinkle et al. have identified the enzyme CaApe2, isolated from the cell wall of C. albicans, and structural and kinetic data support classification as a neutral metalloaminopeptidase arginine/alanine/leucine-specific. However, the authors reported that the enzyme CaApe2 was not able to degrade in vitro collagen type I and IV and that its function is still unknown, but have not ruled out their involvement in the pathogenicity of Candida since CaApe2 can cooperate with proteinases of the host and contribute indirectly in the degradation of collagen. According to the authors, the molecular weight of CaApe2 (107.361 kDa) is significantly higher than the value of 95 kDa metalloproteinase [21].

As proposed by Rodier et al. [20] and Klinke et al. [21], C. albicans metallo proteinase may play a role in fungi spread, since some of them are capable of degrading components of the extracellular matrix, facilitating their access to deeper organs after crossing the endothelial barrier. Thus, metalloproteinases represent a potential target for the development of diagnostic strategies and antifungal drugs based on inhibitors of this metalloproteinase as was demonstrated by Imbert et al. [22, 23] in his two studies.

At long last, the most recent study reports the production, purification, and characterization of a thermostable metalloproteinase from C. kefyr 41 PSB. Furthermore, the authors report that the purified protein appeared as a single protein band at 43 kDa, and in addition, its optimum pH and temperature points were 7.0 and 105 °C, respectively. The protein activity was inhibited using ethylenediaminetetraacetic acid (EDTA), confirming that the protein in question was a metalloproteinase [18].

CONCLUSION

Metalloproteinases secreted by Candida is one factor of pathogenicity of this fungi genus and represents a promising approach to the treatment of candidiasis. However, research in this field needs advancement and improvement in terms of rigor and quality of the studies involved with this theme.

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