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EDITORIAL

Depicting the metabolism of *Paracoccidioides brasiliensis* during infection by transcriptional and proteomic approaches

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Species in the *Paracoccidioides* spp. complex are the causative agents of paracoccidioidomycosis (PCM), considered one of the most important systemic endemic mycosis in Latin America. The genus *Paracoccidioides* comprises 2 species, *P. brasiliensis* and *P. lutzii* with the former being classified into 4 different phylogenetic groups named S1, PS2, PS3 and PS4.[1-3] More recently, the S1 group was sub-divided into 2 lineages (S1a and S1b).[4] The development of the disease depends on several factors dependent on both the host and the fungal virulence. Once the fungal pathogen enters in contact with the host, it should adapt itself to and override the various microenvironmental factors to survive inside the host. Fungal adaptation depends on its metabolism and capability to produce and secrete several molecules that play an important role in virulence. Among these molecules, *Paracoccidioides* species produce enzymes that participate in the most important pathways of central metabolism, involving carbohydrates, lipids, amino acids and nucleotides.[5] In a previous study and using proteomic analysis, it was found that after internalization of *P. brasiliensis* into macrophages, a total of 308 differentially expressed proteins were detected in this fungal pathogen. The upregulated proteins included those related to β-oxidation of fatty acids and amino acids' catabolism, as well as proteins associated with the alternative carbon metabolism and enzymes involved in the gluconeogenesis pathway. As it concerns the downregulated proteins included those related to glycolysis and protein synthesis.[6]

Interestingly, it has been described that most of the *Paracoccidioides* spp enzymes that participate in both the glycolytic pathway and the tricarboxylic acid (TCA) cycle including glyceraldehyde-3-phosphate dehydrogenase (GADPH), enolase (ENO), fructose-1–6-bisphosphate aldolase (FBA), triose phosphate isomerase (TPI), malate synthase (MLS) and isocitrate lyase (ICL), function as moonlight proteins, this is, they are multifunctional proteins capable of accomplishing different functions often unrelated.[7] Thus, these glycolytic enzymes allow *Paracoccidioides* spp binding to extracellular matrix (ECM) proteins such as laminin, fibronectin, fibrinogen, type I and IV collagens and plasminogen, among others. These interactions are involved in the adhesion and invasion process leading to *Paracoccidioides* infection.[7]

Paracoccidioides spp also has the capacity to defend itself against the oxidative and nitrosative stress molecules produced by the hosts immune cells especially those produced by phagocytic cells (mainly macrophages). The oxidative and nitrosative stress are characterized by production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), respectively. ROS comprises superoxide anion radical (O2-•), hydroxyl radical (•OH) and hydrogen peroxide (H_2O_2) while RNS comprises nitric oxide (NO•) and peroxynitrite (ONOO-), among others.[8] These oxidative and nitrosative molecules act on fungal cells through the inactivation of proteins, lipids and cellular enzymes inhibiting respiration, and damaging the DNA and membranes thus leading to the fungal death.[6,9] Therefore, several reports have been shown that *Paracoccidioides* spp has a powerful antioxidant defense system that allows this fungal pathogen to survive ROS and RNS production; this system includes expression/production of several enzymes such as catalases (CATs), superoxide dismutases (SODs), alternative oxidase (AOX), cytochrome c peroxidase (CCP) and thioredoxins (THX).[6,10-13] The important role in virulence of most of these detoxifying molecules has been demonstrated in elegant studies using knock-down or silenced *P. brasiliensis* strains obtained by antisense RNA (aRNA) approaches both *in*

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vitro and *in vivo* assays.[6,10-12] Moreover, Parente-Rocha *et al.* using proteomic analysis after internalization of *P. brasiliensis* into macrophages found upregulation of proteins involved in the oxidative stress response such as SODs, THX and CCP.[6] Of note, all *P. brasiliensis* muted strains obtained through the use of aRNA methodology for SODs, AOX, CATs and CCP, showed diminished survival into macrophages as well as in experimental mouse models.[6,10-12,14]

Most of the vital processes undergone by the eukaryotic cells such as cell cycle, survival, adhesion and proliferation, among other processes, are governed by the signals transduction that in turn are addressed by protein phosphorylation at post-translational levels.[15] Along these lines, Chaves *et al.* investigated the influence of phosphorylative events during the *P. brasiliensis* adaptation to oxidative stress. These investigators used mass spectrometry-based approaches and mapped 440 phosphorylation sites in 230 *P. brasiliensis* proteins finding that phosphorylation at different sites determine the fungal response to oxidative stress.[16]

Several proteases are secreted by *Paracoccidioides* spp with some of them considered as virulence determinants that allow to the fungus to disseminate in its host. Among these molecules several have been described, namely, fructose 1,6-bisphosphate aldolase that binds to plasminogen and activates this into plasmin, which in turn, activates the fibrinolytic activity and also degrades ECM proteins, a fact that enables the fungus to adhere and invade host cells.[17] A secreted aspartyl protease (*Pb*SAP) has also been identified in *P. brasiliensis*.[18] Aspartyl proteases constitute one of the 4 superfamilies of proteolytic enzymes, which have also been found in *Candida albicans* with at least 10 members identified, with SAPs 1–7 being differentially expressed during infection.[19,20] A serine-thiol protease with the capability to degrade laminin, fibronectin, type IV-collagen and proteoglycans, has also been identified in *P. brasiliensis*.[21,22]

Of note, all the above reports using trasncriptomic or proteomic analysis have been performed by means of *in silico* or infected cell lines approaches. In this issue of *Virulence*, the authors of the article entitled "*Paracoccidioides brasiliensis* presents metabolic reprogramming and secretes a serine proteinase during murine infection" developed a method for harvesting *P. brasiliensis* yeasts from the lungs of infected mice to evaluate *in vivo* transcriptional and proteomic profiles.[23] A total of 594 differentially expressed transcripts and 350 differentially expressed proteins were annotated. As described before by the same group,[6] in the investigation presented in this *Virulence*'s issue, authors confirmed the upregulated expression of proteins related to metabolism including glycolysis, detoxifying enzymes and repressed cell wall biosynthesis in *P. brasiliensis*. Moreover, they also confirmed the upregulated expression of a serine protease, an enzyme involved in the invasion and dissemination of this fungal pathogen, shown to be secreted *in vivo* as described by the functional analysis already performed.[22,23] In sum, this article confirmed previous trasncriptomic and proteomic studies with the results presented here providing a better understanding of *Paracoccidioides* spp complex metabolism. Future studies using methodologies to silence or delete specific coding genes to obtain *Paracoccidioides* spp mutant strains, as well as *in vivo* functional studies would reveal key molecules that could be used as targets for developing new therapeutic strategies in PCM.

Disclosure of potential conflict of interest

No potential conflicts of interest were disclosed.

References

[1] Matute DR, McEwen JG, Puccia R, Montes BA, San-Blas G, Bagagli E, Rauscher JT, Restrepo A, Morais F, Niño-Vega G, et al. 2006. Cryptic speciation and recombination in the fungus *Paracoccidioides brasiliensis* as revealed by gene genealogies. Mol Biol Evol 2006; 23:65-73; PMID:16151188; https://doi.org/10.1093/molbev/msj008

[2] Teixeira MM, Theodoro RC, de Carvalho MJ, Fernandes L, Paes HC, Hahn RC, Mendoza L, Bagagli E, San-Blas G, Felipe MS. Phylogenetic analysis reveals a high level of speciation in the *Paracoccidioides* genus. Mol Phylogenet Evol 2009; 52:273-83; PMID:19376249; https://doi.org/10.1016/j.ympev.2009.04.005

[3] Teixeira MM, Theodoro RC, Nino-Vega G, Bagagli E, Felipe MS. *Paracoccidioides* species complex: Ecology, phylogeny, sexual reproduction, and virulence. PLoS Pathog 2014; 10:e1004397; PMID:25357210; https://doi.org/10.1371/journal.ppat.1004397

[4] Muñoz JF, Farrer RA, Desjardins CA, Gallo JE, Sykes S, Sakthikumar S, Misas E, Whiston EA, Bagagli E, Soares CM, et al. Genome Diversity, recombination, and virulence across the major lineages of *Paracoccidioides*. mSphere 2016; 1:pii: e00213-16; PMID:27704050; https://doi.org/10.1128/mSphere.00213-16

[5] Arraes FB, Benoliel B, Burtet RT, Costa PL, Galdino AS, Lima LH, Marinho-Silva C, Oliveira-Pereira L, Pfrimer P, Procópio-Silva L, et al. General metabolism of the dimorphic and pathogenic fungus *Paracoccidioides brasiliensis*. Genet Mol Res 2005; 30:290-308; PMID:16110447

[6] Parente-Rocha JA, Parente AF, Baeza LC, Bonfim SM, Hernandez O, McEwen JG, Bailão AM, Taborda CP, Borges CL, Soares CM. Macrophage interaction with *Paracoccidioides brasiliensis* yeast cells modulates fungal metabolism and generates a response to oxidative stress. PLoS ONE 2015; 10:e0137619; PMID:26360774; https://doi.org/10.1371/journal.pone.0137619

[7] Marcos CM, de Oliveira HC, da Silva Jde F, Assato PA, Fusco-Almeida AM, Mendes-Giannini MJ. The

multifaceted roles of metabolic enzymes in the *Paracoccidioides species* complex. Front Microbiol 2014; 5:719; PMID:25566229; https://doi.org/10.3389/fmicb.2014.00719

[8] Brown AJ, Haynes K, Quinn J. Nitrosative and oxidative stress responses in fungal pathogenicity. Curr Opin Microbiol 2009; 12:384-91; PMID:19616469; https://doi.org/10.1016/j.mib.2009.06.007

[9] Missall TA, Lodge JK, McEwen JE. Mechanisms of resistance to oxidative and nitrosative stress: Implications for fungal survival in mammalian hosts. Eukaryotic Cell 2004; 3:835-46; PMID:15302816; https://doi.org/10.1128/EC.3.4.835-846.2004

[10] Tamayo D, Muñoz JF, Lopez Á, Urán M, Herrera J, Borges CL, Restrepo Á, Soares CM, Taborda CP, Almeida AJ, et al. Identification and analysis of the role of superoxide dismutases isoforms in the pathogenesis of *Paracoccidioides* spp. PLoS Negl Trop Dis 2016; 10:e0004481; PMID:26963091; https://doi.org/10.1371/journal.pntd.0004481

[11] Tamayo D, Muñoz JF, Almeida AJ, Puerta JD, Restrepo Á, Cuomo CA, McEwen JG, Hernández O. *Paracoccidioides* spp. catalases and their role in antioxidant defense against host defense responses. Fungal Genet Biol 2017; 100:22-32; PMID:28093309; https://doi.org/10.1016/j.fgb.2017.01.005

[12] Hernández O, Araque P, Tamayo D, Restrepo A, Herrera S, Mcewen JG, Pelaez C, Almeida AJ. Alternative oxidase plays an important role in *Paracoccidioides brasiliensis* cellular homeostasis and morphological transition. Med Mycol 2015; 53:205-14; PMID:25631476; https://doi.org/10.1093/mmy/myu091

[13] Dantas AS, Andrade RV, de Carvalho MJ, Felipe MS, Campos EG. Oxidative stress response in *Paracoccidioides brasiliensis*: Assessing catalase and cytochrome c peroxidase. Mycol Res 2008; 112:747-56; PMID:18499421; https://doi.org/10.1016/j.mycres.2007.11.018

[14] Ruiz OH, Gonzalez A, Almeida AJ, Tamayo D, Garcia AM, Restrepo A, McEwen JG. Alternative oxidase mediates pathogen resistance in *Paracoccidioides brasiliensis* infection. PLoS Negl Trop Dis 2011; 5:e1353; PMID:22039556; https://doi.org/10.1371/journal.pntd.0001353

[15] Toker A, Marmiroli S. Signaling specificity in the Akt pathway in biology and disease. Adv Biol Regul 2014; 55:28-38; PMID:24794538; https://doi.org/10.1016/j.jbior.2014.04.001

[16] Chaves AF, Castilho DG, Navarro MV, Oliveira AK, Serrano SM, Tashima AK, Batista WL. Phosphosite-specific regulation of the oxidative-stress response of *Paracoccidioides brasiliensis*: A shotgun phosphoproteomic analysis. Microbes Infect 2017; 19:34-46; PMID:27590702; https://doi.org/10.1016/j.micinf.2016.08.004

[17] Chaves EG, Weber SS, Báo SN, Pereira LA, Bailão AM, Borges CL, Soares CM. Analysis of *Paracoccidioides* secreted proteins reveals fructose 1,6-bisphosphate aldolase as a plasminogen-binding protein. BMC Microbiol 2015; 15:53; PMID:25888027; https://doi.org/10.1186/s12866-015-0393-9

[18] Tacco BA, Parente JA, Barbosa MS, Báo SN, Gsóes Tde S, Pereira M, Soares CM. Characterization of a secreted aspartyl protease of the fungal pathogen *Paracoccidioides brasiliensis*. Med Mycol 2009; 47:845-54; PMID:20028235; https://doi.org/10.3109/13693780802695512

[19] Naglik JR, Challacombe SJ, Hube B. *Candida albicans* secreted aspartyl proteinases in virulence and pathogenesis. Microbiol Mol Biol Rev 2003; 67:400-28; PMID:12966142; https://doi.org/10.1128/MMBR.67.3.400-428.2003

[20] Lian CH, Liu WD. Differential expression of *Candida albicans* secreted aspartyl proteinase in human vulvovaginal candidiasis. Mycoses 2007; 50:383-90; PMID:17714358; https://doi.org/10.1111/j.1439-0507.2007.01384.x

[21] Carmona AK1, Puccia R, Oliveira MC, Rodrigues EG, Juliano L, Travassos LR. Characterization of an exocellular serine-thiol proteinase activity in *Paracoccidioides brasiliensis*. Biochem J 1995; 309:209-14; PMID:7619058; https://doi.org/10.1042/bj3090209

[22] Puccia R, Carmona AK, Gesztesi JL, Juliano L, Travassos LR. Exocellular proteolytic activity of *Paracoccidioides brasiliensis*: Cleavage of components associated with the basement membrane. Med Mycol 1998; 36:345-8; PMID:10075506; https://doi.org/10.1080/02681219880000541

[23] Pigosso LL, Baeza LC, Tomazett MV, Rodrigues-Faleiro MB, de Moura VM, Bailão AM, Borges CL, Parente-Rocha JA, Rocha-Fernandes G, Gauthier GM, et al. *Paracoccidioides brasiliensis* presents metabolic reprogramming and secretes a serine proteinase during murine infection. Virulence 2017 [Epub ahead of print]; https://doi.org/10.1080/21505594.2017.1355660