Heme-Induced Ferroptosis Exacerbates Tissue Injury in Disseminated *Candida albicans* Infection

Tanmay Arekar, Divya Katikaneni, Thrisha Acharya, Gabriella Garcia, Michail S. Lionakis, and Yogesh Scindia

Introduction: *Candida albicans* (*C. albicans*) is a commensal fungus whose transition to pathogenicity remains the leading cause of invasive candidiasis, a disease associated with mortality rates approaching 40%. Iron is essential for *C. albicans* growth, and excess host iron worsens outcomes during disseminated infection. During tissue invasion, *C. albicans* damages endothelial cells, creating localized hemorrhagic foci that release cell-free heme from extravascular erythrocytes. This heme serves as a nutrient-rich iron source for the pathogen while simultaneously promoting oxidative tissue injury in the host. Heme is normally neutralized by hemopexin, a hepatocyte-derived heme-scavenging protein, but sustained heme release can overwhelm this protective pathway. The kidney, the primary target organ during disseminated candidiasis, avidly takes up excess heme, generating redox-active iron that can drive lipid peroxidation and ferroptosis, an iron-dependent form of regulated necrotic cell death. We hypothesized that hemopexin deficiency increases renal heme exposure, amplifies ferroptotic injury, and worsens outcomes during disseminated candidiasis.

Methods: Hemopexin-deficient (*Hpx*$^{-/-}$) and wild-type (WT) littermates were infected intravenously with *C. albicans* SC5314, and outcomes were evaluated 3 days later. To determine whether heme alters fungal resistance to innate immunity, *C. albicans* grown with or without heme was exposed to purified neutrophils at increasing multiplicities of infection. Human proximal tubular epithelial cells (PTECs) were exposed to heme and candidalysin to assess renal cytotoxicity. A separate cohort of infected *Hpx*$^{-/-}$ mice received the ferroptosis inhibitor Liproxstatin-2.

Results: Disseminated candidiasis induced progressive heme accumulation in the kidney accompanied by sustained elevations in circulating heme and hemopexin. Compared with WT controls, *Hpx*$^{-/-}$ mice exhibited significantly higher renal fungal burden, enhanced hyphal invasion, greater kidney injury, inflammation, and reduced survival. Infected *Hpx*$^{-/-}$ kidneys contained increased numbers of mature dead neutrophils that resulted in recruitment of CD117^{+} (c-Kit^{+}) immature neutrophils. Lipid peroxidation was detected exclusively in renal tubules of infected *Hpx*$^{-/-}$ mice. Unbiased lipidomic profiling revealed marked triglyceride accumulation, consistent with tubular injury. In vitro, heme-conditioned *C. albicans* displayed increased resistance to neutrophil-mediated killing, while heme directly induced ferroptotic death in PTECs. Treatment with Liproxstatin-2 did not reduce renal fungal burden but significantly attenuated kidney injury and improved disease severity.

Conclusions: Hemopexin protects against disseminated candidiasis by limiting the pathological consequences of excess heme. Heme worsens infection through multiple mechanisms: enhancing fungal growth, increasing resistance to neutrophil-mediated killing, and promoting ferroptotic injury of renal parenchymal cells. These findings identify defective heme sequestration as a previously unrecognized determinant of host susceptibility in invasive candidiasis and reveal heme-driven ferroptosis as a potentially targetable pathway of disease severity. Furthermore, they provide mechanistic insight into the heightened risk observed in patients with liver disease, who produce less hemopexin despite not being neutropenic, and establish a rationale for targeting ferroptosis as an adjunctive therapeutic strategy to complement antifungal treatment in high-risk patients.