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# Struggle for iron: a key attribute for combating Multi Drug Resistance in human pathogen *Candida albicans*

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***Abstract-*** Continuous deployment of antifungals in treating infections caused by dimorphic opportunistic pathogen *Candida albicans* has led to the emergence of drug resistance resulting in cross-resistance to many unrelated drugs, a phenomenon termed as Multi-Drug Resistance (MDR). Although there is reasonable understanding of major factors which contribute to MDR mechanisms, it appears unavoidable to consider novel MDR mechanisms. The ability of pathogenic fungi, including opportunists like *Candida albicans*, to sense and become accustomed to changes in the host environment is essential for their survival and confers the basis of their success as dreadful pathogen. Recent findings suggest that in addition to the classical MDR mechanisms, existence of other novel mechanisms controlling MDR cannot be nullified. Improved knowledge of such mechanisms could facilitate the development of novel therapies to combat these intransigent infections. One such significant environmental factor that *C. albicans* must respond to is iron limitation since they encounter diverse such anatomical sites during the establishment of infection within the host. Considering the importance of *C. albicans* being the fourth most common cause of hospital acquired infectious disease, this review focuses on gaining insights of new regulatory mechanism controlling MDR in *C. albicans* as a response towards iron deprivation.

***Index Terms***- MDR, Candida albicans, iron, microbial infection

## I. Introduction

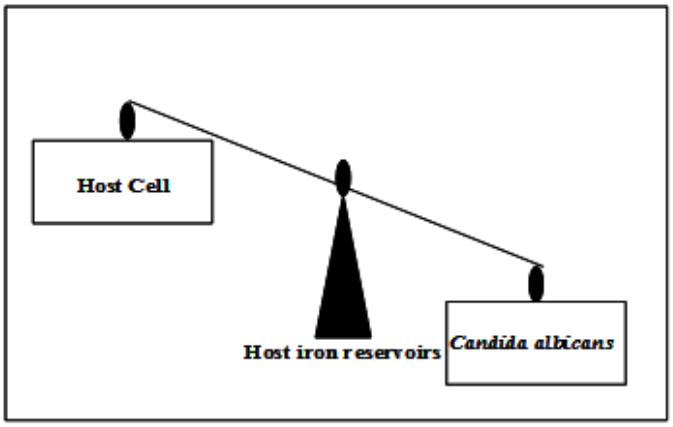
In the last decades, the incidence of fungal infections has increased dramatically due to the rise in the number of immunocompromised patients. The most prevalent fungal pathogen of humans is *C. albicans*. This species ranks as the fourth most common cause of hospital acquired infectious disease and is the primary cause of systemic candidiasis, with mortality rates approaching 50% (Pfaller & Diekema, 2007). The opportunistic pathogen, *C. albicans* is normally a commensal organism in humans, but in conditions such as AIDS, organ transplant, diabetes, or in cancer patients when the host is unable to mount an adequate immune response, it results in mucosal, cutaneous or invasive mycoses (Calderone *et al*., 2002). Continuous usage of antifungal drugs in treating infections caused by *C. albicans* has led to the emergence of drug resistance. This acquired resistance in clinical isolates of *C. albicans* mostly results in cross-resistance to many unrelated drugs, a phenomenon termed as Multi-Drug Resistance (MDR) (Franz *et al*., 1998; Franz *et al*., 1999; White *et al*., 1998). The evolution of drug resistance in fungal pathogens have posed serious problems due to the limited number of clinically useful anti fungal drugs available (Anderson *et.al* 2005; Cowen *et. al* 2008). The various mechanisms of MDR to survive exposure to antifungal drugs includes an over expression or mutations in *ERG11*, encoding the target enzyme of azoles lanosterol 14α-demethylase (Lamb *et al*., 1997; Prasad, 2004; White *et al*.,1998; White *et al*., 2002), an over expression of the drug efflux pumps encoding genes such as *CaCDR1* and *CaCDR2* belonging to the ABC (ATP-Binding Cassette) (Albertson *et al*.,1996; Kohli *et al*., 2002 Sanglard *et al*.,1997) and *CaMDR1* belonging to the MFS (Major Facilitator Super family) transporters (Gupta *et al*.,1998 Pao *et al*., 1998).

In the recent years emerging evidences has demonstrated that there do exists many novel mechanisms controlling MDR and improved knowledge of such molecular mechanisms controlling MDR in pathogenic fungi should facilitate the development of novel therapies to combat these intransigent infections. This review further defines the focus on the urgent need for the understanding of one such novel mechanism regulating MDR i.e. iron availability and attempts to highlight its significance when one considers drug susceptibilities in *C. albicans*.

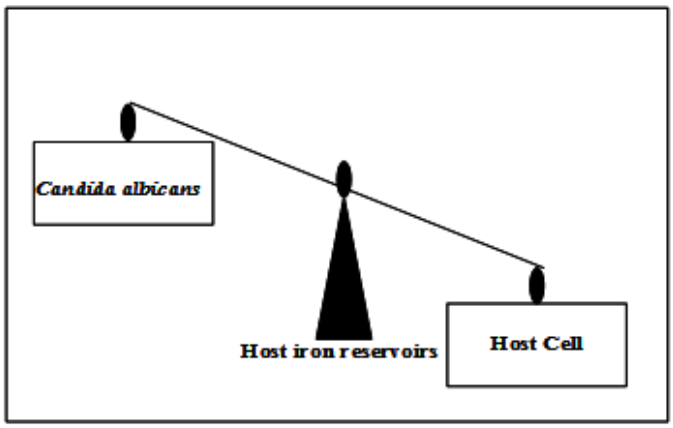
## II. Iron Availability as an Important Biological Determinant Governing Microbial Infection

Intracellular pathogens colonize a variety of anatomical sites which are likely to be scarce in iron, especially under conditions of severe and chronic immunosuppression induced by HIV infection. Apart from exception like *Borrelia burgdorferi* (which does not require iron, Posey & Gherardini, 2000) almost every organisms require iron for growth. Iron is an indispensable critical micronutrient which is required both by the host as well as by the microbial community residing within the host especially as a cofactor in important metabolic functions (Almeida *et al.,* 2009, Bullen *et al*., 2006; Fischbach *et al*., 2006; Nyilassi *et al*., 2005; Spacek *et al*., 2005; Weinberg *et al*., 1999). Iron being a transition metal and because of its ability to donate and accept electrons can participate in the formation of toxic free radicals; therefore, availability of iron in host macrophage cells is tightly regulated (Radisky & Kaplan, 1999). Since iron in macrophages is not freely available, therefore pathogenic organisms needs to exploit host iron reservoirs to make their own

niches within it for their survival. In contrast, macrophages sequester iron to avoid iron acquisition by invading organisms using different strategies (Nairaz *et al*., 2007). They also need to protect iron from intracellular pathogens to maintain their own homeostasis, as iron is required as cofactors in many enzymes including those generating reactive oxygen or nitrogen species as immediate defense against invading organisms. Moreover, the macrophages play critical role in body iron homeostasis by recycling iron for erythropoiesis (Andrews and Schmidt, 2007). So, protecting iron from invading organisms is immensely important for macrophages. This competition between the pathogen and host macrophages for iron represents a critical virulence trait of many infectious diseases (Fig 1) and serves as a natural defense mechanism against infection (Emery, 1980; Kontoghiorghes & Weinberg, 1995; Schaible & Kaufmann, 2004, Weinberg, 1984). Many studies depicting correlations between host iron content and pathogenicity have been observed for the pathogenic fungi *Cryptococcus neoformans* (Barluzzi *et al*., 2002) and *Aspergillus fumigatus* (Kontoyiannis *et al*., 2007) and bacteria such as *Mycobacterium tuberculosis*, Salmonella species and Yersinia species (Schaible & Kaufmann, 2004; Doherty, 2007). Another study revealed the iron dependent role of Leishmania *MDR1* gene in mediating drug resistance (Wong *et al.,* 2006). A recent study shows that iron deprivation downregulates *MDR1*/P-gp function and expression in a leukemic cell line (Fang *et al.,* 2010).




**Survival of Intracellular pathogens**




**Survival of Host Cell**

Figure 1: Iron Balance: Struggle for limited iron reservoirs within the host cells forms the basis of establishment of infection by invading microorganism.

## III. Chemistry and Biology of Iron

The history of earth and evolution of life depicts the abundance, availability, and suitability of iron had been critical to living system. Iron is the fourth most abundant element in the earth crust and second most abundant metal. In the periodic table iron is positioned in the middle. It is a transition metal that exists in various oxidation states ranging however, in the living cell system biochemically, Fe(II) and Fe(III) are the most relevant oxidation states. Transfer of a single electron between these two states is readily accomplished with ascorbate or molecular oxygen. This variability in redox states contributes significantly to the role of iron as an essential biological metal. Although non-toxic in its ferric state, soluble ferrous can be toxic due to its participation in the generation of hydroxyl radicals via Fenton reaction (Halliwell & Gutteridge, 1984). Hydroxyl radicals can depolymerise polysaccharides, cause DNA strand breaks, inactivate enzymes and initiate lipid peroxidation (McCord, 1996).

## IV. Iron Acquisition in C. albicans

Iron acquisition is a critical need for all microorganisms because iron in a human host is not freely available, but mostly linked to host proteins, therefore pathogens such as *C. albicans* must possess mechanisms to gain iron from these proteins. *C. albicans* possesses various type of iron acquisition system, as each system is specifically adopted for a given environmental niche. Here summary of the known and proposed strategies of how *C. albicans* exploits iron from host proteins is mentioned.

***Haemoglobin Uptake:*** Most of the haem in mammalian host is bound to haemoglobin which serves as the largest reservoir of iron for the pathogenic microbes. *C. albicans*, exploit extracellular haemin as an iron source *in vivo* probably through the binding of erythrocyte (Santos *et al* 2003). The iron acquisition by *C. albicans* involves four steps.

a) Binding of haemoglobin to extracellular glycosyl phosphotidylinositol-linked receptor, Rbt5.
b) Endocytosis and probable dissociation of the heme from the globins in the acid environment of the endosome.
c) Degradation of iron-protoporphyrin IX by heme oxygenase, CaHmx1, to alpha-biliveridin, free iron and CO and
d) Uptake of the released iron by the vacuolar iron permease (Weissman *et al* 2008).

***Siderophore Uptake:*** Siderophores are low molecular weight 1.5kda ferric iron chelators that are produced through short, well defined metabolic pathways by many bacteria and fungi under conditions of extreme iron stress (Howard, 1999). These molecules contain lateral chains and functional groups which confer a strong affinity for iron. Most of the fungal siderophores are hydroxamates in nature containing an N-δ-hydroxyornithine

moiety. The uptake of iron siderophore complex is followed by intracelliler reduction of this siderophore bound iron by vaculor enzymes. In addition to being a means of iron aqusition, especialised intracellular siderophore has also been reported to act as iron storage molecule in *A. fumigates* (Howard, 1999; Schrettl *et al* 2008). Although *C. albicans* have not been reported to synthsize and secrete siderophores, they do possess a well defined siderophore uptake system comprise of siderophore transporters such as Sit1p and Arn1-3 hydroxamate transporters (Lesuisse *et al* 1998; Heyman *et al* 2002) indicating that it probably extract iron from siderophores secreted by other organisms in the environment.

***Reductive Iron Uptake:***

This pathway relies on the extracellular ferric reductases (Fre) located in the plasma membrane that can reduce either free or complexed ferric ($Fe^{3+}$) ions into soluble ferrous ($Fe^{2+}$) ions. Since reduced ferrous iron generated by surface reductase activity can be toxic, due to the spontaneous generation of free radicals, $Fe^{2+}$ ions are subsequently oxidized $Fe^{3+}$ and transported into the cell by the protein complex consisting of a multicopper oxidase and iron permease. Therefore $Fe^{2+}$ ions are then taken up by high affinity permeases (Ftr1p) along with the cell surface multicopper ferroxidase (Fet3p) after reoxidation (Askwith *et al* 1994, Stearman *et al* 1996).

An intracellular copper transporter Ccc2p is essential for function of the reductive pathway (Weissman *et al.,* 2002) as it delivers copper to Fet3p and is regulated by cellular iron levels. Multiple homologs of *S. cerevisiae* FRE and FTR genes have been reported in *C. albicans* (Kornitzer, 2009; Baek *et al.,* 2008). Two proteins with iron permease activity are encoded by two highly homologous genes, with different affinities for iron ions, and with different regulation. The high affinity iron permease gene *FTR1* is induced upon iron deprivation, and low affinity *FTR2* is induced when higher levels of iron are available. Moreover, from all components of the reductive pathway, only *Ftr1* has been shown to be crucial for *C. alibicans* virulence in an experimental animal model of infection (Ramanan and Wang, 2000).This reductive iron acquisition system is utilized to scavenge iron from the environment and from high affinity host iron binding proteins such as transferring and ferritin. It has been hypothesized that release of iron from ferritin occurs by acidification of the surrounding environment followed by uptake of iron through reductive high affinity mechanism. Likewise *C. albicans* has also been found to scavenge free iron from transferring via reductive pathway consisting of ferric redcutase, Fre10 and high affinity permease, Ftr1 (Knight *et al.,* 2005).

## V. Starving the invaders to combat MDR

Iron plays a key role in providing natural resistance to infections in humans (as reviewed above). Iron, a ubiquitous redox-active element, serves as a co-factor for several essential enzymes and biochemical processes that includes cellular respiration and metabolism, oxygen transport, drug metabolism and DNA synthesis (Welch *et al*., 2001). In pathogenic *C. albicans*, iron deprivation represents one of the crucial environmental stress conditions it encounters during infection process. Iron acquisition during infection is considered as virulence attribute therefore, the susceptibility to *C. albicans* infections is influenced by iron content of the host. For example, pretreatment of endothelial cells with the iron chelator phenanthroline reduces damage by *C. albicans* due to reduced invasion (Fratti *et al*., 1998). Similarly, loading the epithelial cells with iron increased damage by *C. albicans*, in contrast to the pretreatment of oral epithelial cells with the iron chelator (BPS) (Almeida *et al*., 2008). In a mouse model of systemic candidiasis, an intravenous injection with colloidal iron (60mg per kg body weight) for 3 consecutive days before intravenous inoculation of *C. albicans* yeast cells ($10^7$ cells) significantly increased the mortality rate of mice: within 28 days of infection, 40% of mice without iron administration died, while 80% mortality was observed among iron-loaded animals (Abe *et al*., 1985). Similarly, many other studies have already established roles for iron in epithelial invasions (Heyman *et al*., 2002), infections in mouse model (Ramanan & Wang, 2000) suggesting iron to play a vital role in the virulence of *C. albicans*. Recently role of iron in recurrent vulvovaginal candidosis (RVVC) showed that iron is not only important for pathogenic yeast, but also for normal function of host immunity (Spacek *et al.,* 2005). Kuipers *et al.,* 1999, had shown that lactoferrin, an iron binding glycoprotein, is synergistic with antifungals against different *Candida* species. However, no report was demonstrated whether iron affects drug susceptibility of *Candida* cells experimentally until Prasad *et al.,* 2006, reported for the first time. In the study, they focused on whether availability of iron could have an impact on defense mechanisms of *Candida* against antifungal drugs. Interestingly, it was observed that iron deprivation enhanced drug susceptibility of *Candida* cells resulting in an increase in membrane fluidity, which in turn leads to enhanced passive diffusion of drugs. A link between changes in membrane fluidity to lowered ergosterol levels was established in iron deprived *Candida* cells probably due to down regulation of *ERG11*. Furthermore, Hameed *et al.*, 2011, provided the first novel insight into the intricate relationship between cellular iron, calcineurin signaling, membrane lipid homeostasis and drug susceptibility of *Candida* cells. Recently, Dhamgaye *et al.*, 2012, demonstrated that antifungal action of malachite green is mediated viz depletion of labile iron pools as one of it mechanism. The combination of lactoferrin with fluconazole has been reported to synergistically enhance the antifungal activity of fluconazole against *Candida* spp. (Kobayashi *et al.*, 2011). A significant recent finding establishes that Cap2/Hap43 is essential for *C. albicans* growth under iron-deprivation conditions and for virulence in mouse (Singh *et al.,* 2011). One of the target based approach that has been taken in recent times is the usage of fungicidal monoclonal antibodies which interferes with iron acquisition in *C. albicans* (Brena *et al.,* 2011). Hsu *et al.,* 2011, provided the first evidence that Hap43 is essential for the growth of *C. albicans* under low-iron conditions and for *C. albicans* virulence in a mouse model of infection.

## VI. Iron regulatory circuit links with other MDR mechanisms in C. albicans

Like iron deficiency, *C. albicans* also often encounter sites within the host during infection which has inadequate vascularization and irregular blood flow thus representing

hypoxic areas. Iron limitation appears to limit metabolism largely by similar mechanisms compared with hypoxia as iron proteins are involved in oxygen-dependent reactions. For instance, the activation of *HIF1* is also dependent on cellular iron status as it was shown that the incubation of mammalian cells with iron chelators also activate *HIF-1* (Buss *et al.,* 2004, Comerford *et al.,* 2002,) which in turn activate its target genes like *MDR1*. Interestingly, recent studies suggest that there could be a correlation between intracellular iron and MDR phenomenon in mammalian cells (Epsztejn *et al.,* 1999). Similarly, pH is another environmental condition which *C. albicans* has to cope up within the host. That environmental pH can have influence on the other MDR regulatory mechanisms is amplified by the fact that at alkaline or neutral pH most of the iron is in insoluble ferric form and thus represents a form of iron deficiency. This is also evident from the fact that transcriptome analysis under alkaline pH conditions reveals that a host of upregulated genes were responsible for iron acquisition indicating that *RIM101* pathway also governs expression of iron acquisition genes (Bensen *et al.,* 2004). Moreover, *RIM101* regulated Als3p known to be important for adhesion was recently shown to bind ferritin and mediates growth on ferritin as an iron source (Almeida *et al.,* 2008).

## VII. CONCLUSION

That iron availability is an important aspect which represents a well regulated novel MDR mechanism, therefore, any iron depleting strategy having influence on changes in drug susceptibilities of pathogenic microorganisms including *Candida* either alone or in combination with various antimicrobial compounds certainly merits a closer look. The importance of studying these novel mechanisms in *C. albicans* will help to understand how they are adapted to the mammalian host and potentially identify new approaches for treating infections.

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