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RESEARCH ARTICLE

GLUTATHIONE AND ITS IMPLICATIONS DURING *CANDIDA* INFECTIONS IN HUMANS.

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Abstract

Candida spp. is known for their commensal behavior in humans and animals and studies involving the role of this fungus in adaptation and interaction with the host are also well known. Sometimes, in immunocompromised patients, this *Candida* (*Candida* albicans and *Candida* glabrata) causes superficial and systemic infections in humans known as mycosis and candidiasis respectively. During pathogenesis, *Candida* infect the host cells and evade the host immune response, while the host phagocytes secrete reactive oxygen species (ROS), reactive nitrogen species (RNS), hydrolytic enzymes and antimicrobial peptides to combat and eliminate these pathogens form the host cells. Moreover, antifungals are also known to induce ROS. In response to these oxidant killing pathways of host, *Candida* has developed antifungal strategies to maintain redox homeostasis and escape the antifungal response of the host. Glutathione pathway has been known as the central pathway in maintaining redox balance in yeast. The recent literature has shown an increasing trend in connecting the antifungal response with redox homeostasis in fungal pathogens. In this review, we present an updated knowledge about the recent findings in glutathione pathway involved in *Candida* survival and pathogenesis.

Introduction:-

Candida albicans is the major cause of fungal infections followed by *Candida* glabrata in normal as well as immunocompromised patients. However, the fungal infections, mycosis or candidiasis can prove lethal to patients with weak immune system (Luis A. Pérez-García, 2017). The only treatment available to treat these infections is the use of antifungal drugs belonging to class, azoles and echinocandins (Odds et al., 2003). The uncontrolled use of these antifungals has been associated with the drastic rise in nosocomial fungal infections involving drug resistant strains (Wiederhold, 2017). The antimicrobials in general including antifungal drugs have been implicated to interfere with the redox homeostasis in *Candida*. The antifungal induced redox imbalance induces signaling mechanism to induce the antioxidant response and activation of transporters to efflux the antifungals from the cell to outside (Cannon et al., 2009; Cannon et al., 2007). Glutathione (GSH) is the main low molecular weight thiol present in all living organisms—bacteria, fungi, animals and plants (Lushchak, 2012). This thiol is required by all organisms as a central regulator to maintain redox homeostasis of intracellular environment (Lushchak, 2012; Sies, 1999; Wu et al., 2004). GSH is a tripeptide formed by the three amino acids glutamate, cysteine and glycine with a free thiol group. During oxidative stress, GSH acts as an electron donor to reduce the oxidized proteins, and inturn gets oxidized to form GSSSG by linking of two GSH molecules by as disulphide bond. This oxidized GSH (GSSG) is reduced and recycled back to reduced GSH (GSH) by a redox regulated enzyme known as glutathione reductase

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(Grx) using NADPH as a cofactor. GSH is the main thiol to maintain redox state of the cell, disturbance in this pathway is implicated in the prognosis of many diseases.

The strategies to combat redox stress and thrive in the oxidative environment inside the host phagocytes and during antifungal drug treatment play a major role in the success of fungal pathogens *C. albicans* and *C. glabrata* (Briones-Martin-Del-Campo et al., 2014; Enjalbert et al., 2007). GSH is also responsible for export of antifungal drugs and xenobiotics from the cells by conjugation with these species and effluxing using ATP driven transporters (Cannon et al., 2009). This review will give an in-depth knowledge of glutathione pathway, biosynthesis, its role in *Candida* pathogenesis and antifungal drug response.

Glutathione Biosynthesis and regulation:-

GSH being major thiol to maintain the redox state should be continuously made and the level should be tightly regulated inside the cell. GSH biosynthesis is a two step reaction catalyzed by two cytosolic enzymes from its constituent amino acids. One of the enzymes is γ-glutamylcysteine synthetase (GCS1), also known as glutamate-cysteine ligase catalyzes the first step of ligating glutamate and cysteine to form a dipeptide γ-glutamylcysteine. In the second step, the γ-glutamylcysteine formed in previous reaction is ligated to glycine in an ATP driven reaction catalyzed by GSH synthetase (GSH2) (Penninckx, 2002). The estimated concentration of GSH present in yeast cells is approx.10 mM. The low redox potential (E'(o)=-240 mV for thiol disulfide exchange is the main reason for its redox buffering properties in the living cells by acting as a strong electron donor for proteins and oxidants. GCS1 has been reported to be essential in *C. glabrata*, while non-essential for *C. albicans*. Interestingly, in *C. albicans*, GCS1 has been implicated to be essential for survival in macrophages and murine model of infection (Yadav et al., 2011). This observation can be attributed to the fact that the dipeptide γ-glutamylcysteine has been able to substitute the functions of GSH to reduce the oxidized macromolecules of the living cells (Grant et al., 1997). Yap1 is a redox sensitive transcription factor regulating many antioxidant genes in yeast. It usually acts by activation under the control of Met4 transcriptional factor or regulated by thoredoxin. On depletion of GSH in cells, Met4 gets activated and leads to Yap1 induced expression of GSH1 catalyzing the rate limiting step in GSH biosynthesis. Interestingly, during the addition of methionine, Met4 gets ubiquitinated, resulting in the repression of this enzyme and leads to depletion of GSH in the cells. Met4 has no DNA binding domain but it is associated with Cbf and Met31/Met32 binds to gsh1 gene and induction occurs under supfur limitation and under cadmium stress. Yap1 dependent induction in GCS1 and GSH2 genes under oxidative stress is dependent on thioredoxin. Thioredoxin under normal conditions is in reduced state in the cells and hence keeps the Yap1 deactivated. Under the oxidative stress conditions, thioredoxin gets oxidized and activates Yap1- a bZip transcriptional factor inducing the GSH biosynthesis genes and increases GSH in cells to combat oxidative stress (Trotter and Grant, 2003; Wu and Moye-Rowley, 1994). GSH depletion has been also illustrated to be the cause of yeast to hyphae transition in *Candida albicans*. The addition of reducing agents exogenously in the medium like dithiothreitol can render the cells viable and inhibit the yeast to hyphae transition (Wheeler et al., 2002; Wheeler et al., 2003). GSH levels increase dramatically under the conditions of oxidative stress, presence of xenobiotics, under sulfur and nitrogen limitation, addition of toxic metabolites, heavy metal exposure and yeast to hyphae transition.

Glutathione system and its associated redox sensitive proteins:-

Following the synthesis of GSH in the cytoplasm of cells, GSH is transported to other subcellular compartments via GSH transporters. GSH protects these environments from oxidative damage and regulate the proteins essential for smooth functioning of some important cellular responses, e.g. autophagy, apoptosis, cell cycle, etc. The GSH dependent redox regulated proteins are glutathione peroxidase (Gpx) and glutathione transferase (Gst). Gpx is involved in detoxification of hydrogen peroxide and lipid peroxides and Gst mediates the conjugation of GSH with toxic metabolites and export them to outside through transporters. Oxidized proteins accept electrons from GSH to form an intermediate mixed disuphide in a process known as gluthionylation or glutathionation. This mixed disulphide is then resolved to recycle GSH and reduced protein by another enzyme called glutaredoxin (Grx). In another mechanism, reduced Grx donates electrons to oxidized proteins and inturn gets oxidized. This oxidized Grx is recycled back by GSH to form reduced Grx again, in turn oxidizing GSH to GSSG. GSSG is then reduced back by glutathione reductase (GR) to recycle GSH in the cells as shown in Fig. 1.

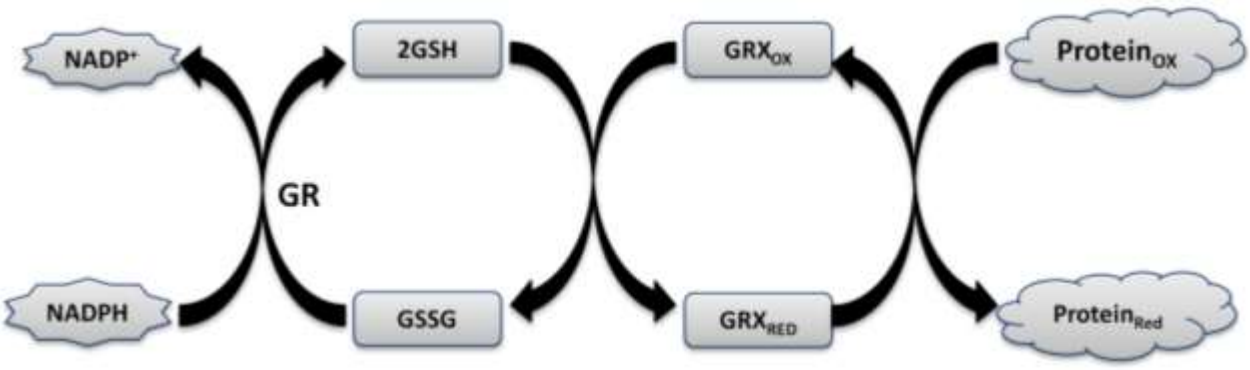

Fig. 1:- Schematic representation of glutathione system in action during oxidation of protein under oxidative stress. The oxidized disulphide form of proteins gets reduced by glutaredoxin (Grx) inturn getting oxidized. Oxidized glutathione is reduced by reduced GSH to form oxidized GSH (GSSG). This GSSG is reduced back to GSH by glutathione reductase (GR) in NADPH dependent manner.

Regulation of antifungal responses by glutathione system:-

GSH is considered essential in eukaryotes unlike in prokaryotes that can grow in minimal media in absence of GSH (Greenberg and Demple, 1986; Wu and Moye-Rowley, 1994). *Candida albicans* and Candia glabrata on entering the host cells encounter the higher concentration of ROS and RNS, resulting in damage to macromolecules, ultimately killing the pathogens. Glutathione pathway is one of the major pathways to maintain redox homeostasis during stress conditions in the cell. The redox regulated responses in *Candida* spp. are still in infancy and it is difficult to say what could be the outcome of GSH perturbations in cells under stress. However, there are a good number of studies carried out to dissect the redox regulated pathway in *Candida* pathogens.

UV irradiation and antimicrobial drugs induce DNA repair response in mammalian cells by producing ROS in mitochondria dependent manner (Benhar et al., 2001; Kalghatgi et al., 2013). ROS is also produced in microbes by microbicidal antimicrobials, and is considered one of the major factors to kill the microbial pathogens. Although, the ROS induction by antibiotics has been illustrated in many landmark studies by different labs, but the topic is still very debatable contradicted by many other labs (Dwyer et al., 2014; Dwyer et al., 2009; Fang, 2013; Van Acker and Coenye, 2017). Recent literature has also demonstrated that the antifungal drugs induce ROS as part of their killing spree in *Candida* spp. To counteract the killing by ROS, *Candida* has evolved some antioxidant proteins surface SODs, peroxidases, glutaredoxins and glutathione peroxidases. There is not much progress in the development of methods for the accurate measurement of GSH or GSSG in fungal cells. The difference in the GSH/GSSG in fungal cells during drug treatment would be a good insight in the involvement of ROS in drug resistance. In *C. albicans*, the drug treatment leads to induction of ATP-binding cassette (ABC) membrane transporters. The drugs form conjugates with GSH and are effluxed out of the cell. The resistant strains have higher induction of these transporters(Wiederhold, 2017). Multidrug resistant (MDR) *Candida* spp. have overproduction of the ABC transporter (also known as major facilitator superfamilies (MFS) (Hiller et al., 2006; Niimi et al., 2004). Interestingly fluconazole (an azole) and micafungin (an echinocandin) have been shown to interfere with the intracellular redox state of the cells, with the maximum impact on glutathione metabolism. Interestingly, *C. albicans* strains that are resistant to fluconazole have induced levels of glutathione to counter the ROS induced by these drugs. Micanozole kills majority of fungal cells by induction of ROS. The antioxidant, pyrrolidinedithiocarbamate (PDTC), at 10 µM increased the MIC 1.25 µg/ml to 12.5 µg/ml. In *C. albicans*, GCS1 is non-essential in vitro but without this gene, the survival of *C. albicans* is questioned. It also illustrates that the GCS1 is also required for survival in murine model of infection. Interestingly, in *C. glabrata*, GCS1 is very much essential for survival in vitro also probably due to lack of GSH transporters (Yadav et al., 2011). Horseradish essential oil (HREO) is a mixture of different isothiocyanates having a antifungal activity against *Candida albicans* is also acting by production of ROS. In *C. albicans* it leads to depletion of GSH and increase in hydrogen peroxide, damaging the cells (Bertoti et al., 2016; Yadav et al., 2011). Furthermore, Ebselen; a mimic of GSH peroxidase has been shown recently to have antifungal activity against *Candida* by depletion of GSH and increase in ROS in cells (Thangamani et al., 2017). Hence, GSH system in *Candida* is playing some crucial role in providing resistance to antifungal drugs.

Conclusion:-

The main regulator of cellular redox state is GSH pathway maintaining the redox homeostasis in yeast cells. Moreover, the antifungal drugs have been shown to induce ROS as a part of their strategy to kill the fungal cells. GSH being the redox buffer plays an essential role in the survival and establishing infection inside macrophages and in murine model of candidiasis. The essentiality of GCS1 or GSH1 has been demonstrated by many studies and featured as a drug target for *Candida*. Future research should be focused on establishing the relationship between ROS and antifungal drug mechanism to reveal the new insights about the involvement of GSH pathway to combat the antifungals.

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