GLUTATHIONE AND ITS IMPLICATIONS DURING CANDIDA INFECTIONS IN HUMANS.

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Abstract

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Glutathione (GSH) is the main low molecular weight thiol present in all living organisms—bacteria, fungi, animals and plants (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 GSSG by linking of two GSH molecules by a disulphide bond. This oxidized GSH (GSSG) is reduced and recycled back to reduced GSH (GSH) by a redox regulated enzyme known as glutathione reductase.

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 antifungals are also known to induce ROS. In response to these antifungal induced oxidative stress, 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 yeasts. 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.
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 disulfide in a process known as glutathionylation or glutathionation. This mixed disulfide 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 intrum 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.
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 Candida 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. Micanazole kills majority of fungal cells by induction of ROS. The antioxidant, pyrrolidine-dithiocarbamate (PDT), 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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