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REVIEW ARTICLE

EPIGENETIC MECHANISMS INVOLVED IN PHASE SWITCHING PROCESS EXHIBITED BY *CANDIDA ALBICANS*.

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Abstract

Candida albicans is a benign member of the normal skin and mucosal flora. The “frenemy” can cause life-threatening illness in an immunocompromised host, hence termed an opportunistic pathogen. The spectrum of disease caused by *Candida* may range from a simple diaper rash seen in infants to systemic candidiasis in frail patients. Mortality rate of systemic candidiasis reported in different cases were found to be 30-50%. *Candida albicans* exhibit extreme morphological plasticity which is a vital factor for its virulence. The transition from pseudohyphae to hyphal forms promotes tissue invasion and penetration into tissues. Over the past several decades researchers have worked on several virulence factors of *Candida albicans* to dissect the mechanism of pathogenicity in the host. The proteome, transcriptome and the genomic analysis have provided insight into the fundamental mechanisms of *Candida* mediated virulence and pathogenicity. Now they have embarked on revealing the epigenetic tags, which has a profound effect on the virulence of *Candida*. Epigenetics marks do not involve DNA sequence rather they influence gene expression by modifying the proteins or factors involved in the regulatory process. Hence, this review will throw light on the epigenetic process associated with phase-switching in *Candida albicans*. The process of phase switching happens to be a novel and less debated mechanism, which forms the crux of this review. Although there are several reports on in vitro and in vivo studies on animal models related to phase switching, this review would principally focus on the process in connection with oral cutaneous candidiasis.

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Candida the frenemy within:-

Candida albicans is an extraordinarily versatile opportunistic pathogen and is considered as a harmless commensal of mucosal surfaces. When the equilibrium of these surfaces is disturbed within the host, frequent and recurrent candidiasis is established (Sobel et al., 2003 and Edmont et al., 1999). Several properties like tissue adherence, iron sequestration, phenotypic switching and reversible transition of blastospore and extended filamentous forms play a vital role in virulence of *C.albicans* (Caldirone and Fonzi, 2001). Various physical and genetic factors also play a vital role in phenotypic switching leading on to virulence of *C.albicans*. Involvement of gene pools and several signal transduction pathways controlling morphogenesis had been implicated (Ernst et al., 2000, Liu et al., 2002). Also, regulators of morphology specific gene transcription are identified in virulence strains and a growing

appreciation of the importance of epigenetic mechanisms in the gene regulation of phenotypic switching has emerged in recent decades (Grant Downton and Dickinson, 2006). Indeed, among the various epigenetic mechanisms like DNA methylation, phosphorylation, ubiquitination, centromeric chromatin alterations, histone acetylation leads its role in phenotypic switching (Klar et al., 2001). With this background, this review throws insight on the novel mechanism of epigenetic phase switching prevailing among the commensal *C.albicans* in mucocutaneous tissues transforming it as an opportunistic pathogen: the “frenemy” within the host.

Major virulence factors:-

The factors responsible for initiation, propagation and sustenance of disease in host need to be clearly defined in the case of an opportunistic pathogen like *C.albicans*. In this context, an opportunistic pathogen should accomplish the following criteria: (a) component which damages host should be present in the pathogen, (b) the pathogen should exhibit all traits to establish disease,(c) factors present in the pathogen should directly interact with host cells. *C.albicans* adapts to host niche by inducing transcriptional and translational changes which enhances its survival under host environmental conditions. Several specialized mechanisms have to be activates so as to promote the establishment, dissemination and pathogenesis (Brock, 2009). Thus, specialized adaptations of *Candida* to changing host microenvironments aid in sustaining infection in the host. The major factors which contribute to the pathogenesis and virulence of *Candida* are mentioned in Table 1.

Table 1: Major virulence factors associated with the virulence of *Candida albicans*.

PROPERTY	FUNCTION	GENES INVOLVED
ADHESION	Adherence to cells	als1p, 3p, 4p, 5p, 6p, 7p, 9p
MORPHOGENESIS	Phase transition (pseudohyphae to hyphae)	phr1, ece1, hyr1, rbf1, chs2, chs3
PHENOTYPIC SWITCHING	White-opaque switching	wor1, wor2, efg1
PHOSPHOLIPASES	Tissue invasiveness	Plb1, Plc
PROTEINASES	Degradation of tissue barriers	sap1, 2, 3, 5, 6, 9
BIOFILM FORMATION	Adherence onto medical devices	bcr1, hwp1, sun41, nup85, mds3, kem1

Morphogenesis and phase switching:-

The transition of *C.albicans* from unicellular yeast form to filamentous form (hyphae or pseudohyphae) is termed as morphogenesis. *C.albicans* and *C.dubliniensis* are the only species of the genus which can undergo morphogenesis. The process of morphogenesis is enabled by the presence of an array of factors including nutrients, near-neutral pH, a temperature range of 37 – 40°C, CO₂ concentration of 5.5%, presence of N-acetyl D-glucosamine, serum, amino acids and biotin. The reverse of this process produces yeast forms from hyphal forms which is accelerated by low temperature, acidic pH, absence of serum and high concentration of glucose (Corner and Magee, 1997; Eckert et al., 2007). The mechanism of transition morphogenesis is vital for pathogenesis as the yeast forms are suited for propagation in tissues, whereas hyphae are required for tissue invasion and damage.

Table 2: Major differences between the process of morphogenesis and phase-switching in *Candida albicans*.

PROPERTY	MORPHOGENESIS	PHASE-SWITCHING
PHENOTYPE	All cells in a population exhibit the same phenotype.	A group of cells in the population switch phase or show a different phenotype.
TRANSITION FREQUENCY	High	Low (less than 3%)
TEMPERATURE SENSITIVITY	Mycelia develops at lower temperature (34°C)	Cell switching happens at (25°C)
PRESENCE OF OXYGEN	Requires microaerophilic conditions	Rapid switching at anaerobic conditions
FAVORABLE MECHANISM	Pathogenicity Invasion & tissue damage	Adaptive Mating
EPIGENETIC FACTOR	DNA methylation	Histone deacetylation

White-Opaque cells (W-O cells):-

C.albicans possess a unique property to switch between two specific types of cells, white and opaque, which is not found even in closely related species. The process of white-opaque switching was discovered by David Soll and Colleagues in 1987 (Slutsky et al., 1987). It was only after a decade or so the principle role of phase switching in the mating cycle was established. Each cell type can propagate for many generations without any change in their genome sequence. About seven percent of the genome, coding for about 400 genes are known to regulate the phase switching process (Magee and Magee, 2004; Bennett and Johnson, 2005). The properties of white and opaque cells have been tabulated in table 3.

Table 3: Characteristics of white and opaque cells.

PROPERTY	WHITE	OPAQUE	REFERENCE
CULTURAL CHARACTERISTICS	Spherical and produce shiny, domed colonies under	Elongated and produce flatter, darker colonies	Slutsky, et al., 1987
MATING ABILITIES	Less competent/No competence	10 ⁶ times more competent than white cells.	Miller, et al., 2002
METABOLIC PREFERENCES	Fermentative	Oxidative	Lan, et al., 2002
INTERACTION WITH HOST IMMUNE CELLS	More resistant to killing by PMNR cells	Sensitive to ROS, produced by phagocytic cells	Sasse, et al., 2013
INVASION	Invasive - Systemic	Highly invasive in cutaneous model	Kvaal, et al., 1999

The difference in phenotype and transition from white to opaque forms contributes to the virulence of the organisms in different biological environments. Kvaale et al., in 1997 provided evidence for the niche specific transition of W-O cells. When mice were infected with opaque type of cells, only a few cells could be recovered from their kidney which was the target organ in the infection model, when compared to white cells. The results indicated that most of the cells switched from opaque to white type which conferred a selective advantage under the host conditions. In contrast to the above study, opaque cells colonized the skin of mice more effectively than white cells in a cutaneous infection model. Protease secreted by opaque cells was attributed for this virulence property. Hence, conditions prevailing in the host decide the transition of W-O cells, making it advantageous for colonization or dissemination of infection (Kvaale et al., 1999).

Mechanism of phase switching (PS):-

The process of phase switching is strictly regulated by the transcriptional regulators encoded at the Mating type-like locus (MTL). Two different alleles, MTL α and MTL α exist in majority of *C. albicans* strains. Cells heterozygous for MTL locus produce a heterodimer $\alpha 1-\alpha 2$ which is found to be a major repressor of phase switching. However, in conditions leading to loss of heterozygosity (LOH), either by mitotic recombination or loss of one copy of chromosome 5 containing one allele and duplication of the other homolog, the cells turn out to be either MTL α or MTL α (Wuet al., 2005, 2007). These cells lose their ability to produce the heterodimer and hence become switching competent. The cells thus produced are stable for several generations under normal laboratory conditions. This property is attributed to the transcriptional circuit consisting of several interlocking feedback loops. They tend to regain their potential to synthesize heterodimer after mating of a α and α producing a tetraploid cells, which in turn aids the cells to switch phase. The phase switching mechanism does not involve changes in the DNA sequence but is chiefly controlled by epigenetic factors (Bennett and Johnson, 2003) (Figure 1).

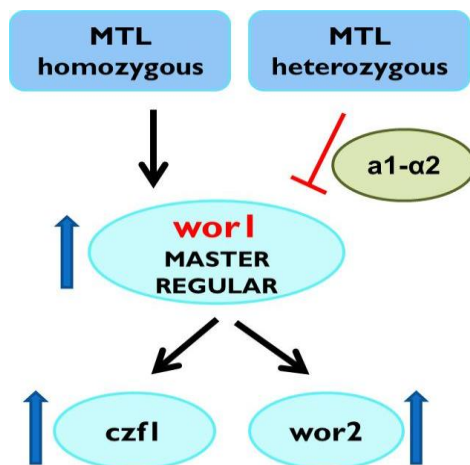


Figure 1:- Mechanism of phase switching in *Candida albicans*.

The molecular mechanism underlying the transcriptional regulation and the feedback loops involved have been elucidated by (Zordan et al., 2007). Wor 1, (White-Opaque Regulator) a protein that is the master regulator of switching process is required for establishment and maintenance of opaque form. The expression of Wor 1 is found to be low in white cells, in contrast, the opaque cells induces autoactivation of Wor 1 gene, hence maintaining an increased concentration of Wor 1 protein (Huang et al., 2006; Srikantha et al., 2006). Consequently, Wor 1 activates two other genes called Czf1 (*Candida albicans* Zinc Finger 1) and Wor 2, where Wor 2 directly activates the expression of Wor 1, completing the positive feedback loops. The protein Czf 1 indirectly maintains the expression of Wor 1, by repressing the Efg 1 (Enhanced Filamentous Growth), which is responsible for maintaining stable white phase in cells. Taken together, Wor 1, 2 and Czf1 proteins are present at a high concentration in opaque state whereas Efg1 concentration is higher at white phase. The transcriptional regulation through these feedback loops determines the phenotype of the cells (Figure 2) (Sriram et al., 2009).

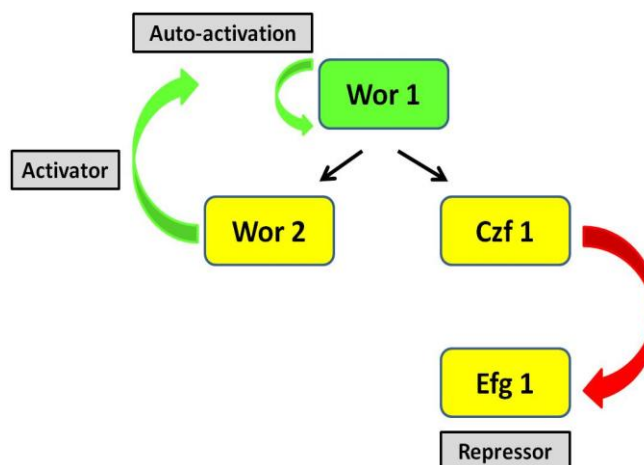


Figure 2:- Feedback loops involved in the maintenance of opaque phase in *Candida albicans*.

Numerous studies have provided evidence on the function of Wor 1 as the key regulatory molecule effecting phase switching process. Artificial or induced expression of Wor 1 from heterologous promoter in *a/a* cells promotes W-O switching, but the mating process is hindered as the genes essential for mating are repressed by *a1/a2* repressor (Huanget al., 2006). Vincet al., 2007, studied the effect of Czf 1 overexpression on switching process, which revealed that even high concentration of Czf 1 could not overcome the repression of Wor 1 by the heterodimer *a1/a2*. Interestingly, the deletion of Efg 1, the repressor of opaque switching, did not show any effect on the phase switching process as the expression of Wor 1 is strongly regulated by the expression of *a1/a2* (Zordan et al., 2007).

Epigenetics tags involved in PS process:-

The phase switching in either direction is modulated by external environmental conditions, which substantiates the involvement of epigenetic factors in the process. Chromatin modification by histone deacetylation has been documented as an important epigenetic mark influencing the transition. Inhibition of histone deacetylation by trichostatin A or deletion of HDA1 gene encoding histone deacetylase, are known to stimulate white to opaque switching. In addition, deletion of RPD3 encoding histone acetylase increased frequency of bidirectional switching (Klar et al., 2001). Cells lacking HDA1 gene or mutants expressing low levels of Hda1 protein shows increased switching to opaque phase, which is paralleled with the reduced expression of Efg1 (Srikantha et al., 2001). Another interesting finding is that the Efg1Δ mutant opaque cells switch to white phase after the repression of Set3, a histone deacetylase complex, which provides evidence on the derepression of Wor 1 protein in the absence of Efg1 is driven by histone deacetylase. Consequently, these experimental evidences prove that Candidal cells sense the biological environment prevailing in the host and adapt themselves to the selective pressure by way of phenotype transition.

Clinical significance:-

The formation of white and opaque cells is an adaptive response exhibited by the yeast cells to escape host defence mechanisms. Although both white and opaque cells are phagocytosed with an equal frequency, white cells exhibit more resistance to killing than opaque cells. Opaque cells are more sensitive to ROS (reactive oxygen species) produced by phagocytes (Kolotila et al., 1990). Interestingly, opaque cells have developed mechanisms to avoid recognition by host defences, while white cells fail to do so (Geiger et al., 2004). A study by Lohse and Johnson, 2008 showed that opaque cells are not effectively phagocytosed when compared to white cells when exposed to two different types of innate immune cells the *Drosophila* S2 and mouse macrophage derived cell line. Hence, it is vivid that the phase switching mechanism in a way aid *C.albicans* to oppose or evade host immune components.

In addition to the above views, the transition state also enables the organism to survive at different biological niches, for example, opaque cells are found to stable at lower temperatures like the skin surface, where as white cells have been recovered from internal organs of infected mice (Kvaal et al., 1999). Biofilm formation and adherence is also facilitated by this transition although the underlying molecular mechanism has not been clearly defined.

Conclusion:-

Candida albicans establishes diverse mucosal and systemic diseases in mucocutaneous tissues in immunosuppressed and immuno-compromised patients. This versatility depends on the co-ordination of gene expression, albeit, epigenetic regulation in *C.albicans* is poorly characterized. In recent decades, among the various virulence factors implicated, phenotypic phase switching seems to be alarming among the treating medical mycologists and geneticists. Transition frequency varies depending on the growth temperature, oxygen levels and adaptive mating mechanisms. Genetic based analysis of phenotypic switching relates this occurrence to DNA methylation and histone deacetylation. More definitive conclusions and understanding of this simple relation between epigenetic mechanisms with phenotypic switching will aid in the achievement of novel therapeutic, preventive and diagnostic targets in *C.albicans*.

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