المستخفية المورمة ومجال الـ F البروتيني

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الخلاصة:

Cryptococcus neoformans هي فطريات تشبه الخميرة تسبب التهابات جهازية ، خاصة في المرضى الذين يعانون من ضعف المناعة. توجد هذه الفطريات في بيئات مختلفة ، مثل الفاكهة والتربة وفضلات الطيور. نوعان رئيسيان يصيبان البشر ، مما يؤدي إلى الإصابة بالمكورات الخفية. الأشخاص الذين يعانون من ضعف في جهاز المناعة ، وخاصة المصابين بالإيدز أو الذين يخضعون للعلاج المثبط للمناعة بعد زراعة الأعضاء ، يكونون أكثر عرضة للإصابة بالعدوى. يؤثر التهاب السحايا بالمكورات الخفية على حوالي 220.000 مريض مصاب بفيروس نقص المناعة البشرية سنويًا ، مما يتسبب في وفاة ما بين 150.000 و 200000 شخص.

Cryptococcus neoformans هو السبب الرئيسي لالتهاب السحايا الفطري والتهابات الجهاز العصبي المركزي ، مما يساهم في حدوث وفيات عالمية كبيرة ، لا سيما في أفريقيا جنوب الصحراء الكبرى. ويؤثر بشكل أساسي على المرضى الذين يعانون من كبت المناعة ، مع معدل وفيات مرتفع يصل إلى 82٪. في حين انخفض معدل الإصابات المرتبطة بفيروس نقص المناعة البشرية في البلدان المتقدمة ، تظل العدوى الانتهازية مصدر قلق كبير في المناطق ذات الوصول المحدود إلى الرعاية الصحية. تم العثور على حالات التهاب السحايا بالمكورات الخفية في الغالب في البلدان المنخفضة والمتوسطة الدخل ، ولا سيما أفريقيا جنوب الصحراء الكبرى. يمكن أن يساعد توافر HAART في تقليل انتشار المكورات الخفية والتهاب السحايا الفطري والوفيات المرتبطة بها. في المرضى غير المصابين بفيروس نقص المناعة البشرية ، قد تزيد العلاجات المثبطة للمناعة من خطر الإصابة بالمكورات الخفية والالتهابات الفطرية الأخرى.

تتكون بروتينات F-box (FBP) من حوالي خمسين حمضًا أمينيًا وتعمل كموقع تفاعل بين البروتينات. تعمل كعناصر كاسحة في الخلايا ، وتجمع البروتينات لإرسالها إلى مجمع SCF.يتكون مجمع SCF من بروتين F-box (FBP) و Skp1 و Rbx1 و Cul1. يلعب FBP دورًا مهمًا في تحديد ركائز ليجازات SCF ، مما يضمن خصوصية عالية من الركيزة. يساهم FBP في العديد من الوظائف الخلوية مثل الساعات البيولوجية والنسخ والتطوير ونقل الإشارات ودورات الخلية واستشعار المغذيات. تنظم هذه البروتينات بشكل انتقائي مستويات البروتين في الخلية عن طريق استهداف بروتينات معينة يتم تعديلها في مركب SCF.

الكلمات المفتاحية : Cryptococcus neoformans ، الضراوة ، المجال البروتيني F- box

Cryptococcus Neoformans And F-Box Protein

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Abstract:

Cryptococcus neoformans are yeast-like fungi causing systemic infections, primarily in patients with compromised immunity. These fungi are found in

various environments, such as fruit, soil, and avian excreta. Two main species infect humans, resulting in cryptococcosis. People with weakened immune systems, particularly those with AIDS or undergoing immunosuppressive therapy after an organ transplant, are at a higher risk of infection. Cryptococcal meningitis affects around 220,000 HIV-infected patients annually, causing 150,000-200,000 deaths. Cryptococcus neoformans is the leading cause of fungal meningitis and CNS infections, contributing to significant global deaths, especially in sub-Saharan Africa. It primarily affects immunosuppressed patients, with a high mortality rate of up to 82%. While the rate of HIV-related infections has declined in developed countries, opportunistic infections remain a major concern in areas with limited healthcare access. Cryptococcal meningitis cases are predominantly found in low and middle-income countries, particularly sub-Saharan Africa. The availability of HAART could help reduce cryptococcal prevalence, fungal meningitis, and associated deaths. In non-HIV patients, immunosuppressive treatments may increase the risk of cryptococcosis and other fungal infections. F-box proteins (FBP) are composed of around fifty amino acids and function as an interaction site between proteins. They act as scavenger elements in cells, gathering proteins to be sent to the SCF complex. The SCF complex consists of F-box protein (FBP), Skp1, Rbx1, and Cul1. FBP plays a crucial role in identifying substrates for SCF ligases, ensuring high substrate specificity. FBP contributes to various cellular functions such as circadian clocks, transcription, development, signal transduction, cell cycles, and nutrient sensing. These proteins selectively regulate protein levels in a cell by targeting specific proteins to be modified in the SCF complex.

Key word : Cryptococcus neoformans , virulence , F- box protein

Introduction

Cryptococcus neoformans are yeast-like fungi that can lead to systemic infections (cryptococcosis), particularly in patients with mediated immunity (1). It isolated cryptococcus from peach juice and subsequently demonstrated pathology in laboratory animals(2) . *Cryptococcus neoformans* is a free-living organism that can exist in many niches worldwide. Fruit and soils were isolated from pigeons and other avian excreta(3). *C.neoformans* an opportunistic Basidiomycota (4,5) Phylum pathogen with the two most frequently known human-infected species, *Cryptococcus neoformans*, consisting of cryptococcal serotypes D and A, and *Cryptococcus neoformans*, consisting of serotypes B and C (6,7). Most cryptococcal patients especially CD4+ lymphocytes, are immune-mediated. AIDS poses a significant risk factor of 15-20% in the U.S. and 55-70% in Latin America and Sub-Saharan Africa (8). Another big risk factor in solid-organ transplant patients is immunosuppressive therapy (9). Current

cryptococcal meningitis with about 150,000-200,000 deaths per year (11), is estimated at around 220,000 cases a year for HIV-infected patients (10) .

The most prevalent cause of fungal meningitis and CNS (central nervous system) infection is *Cryptococcus neoformans* (12). It contributes to significant annual world death, particularly in sub-Saharan Africa (9, 13). It especially affects immunosuppressed patients (14) with a reported mortality of up to 82% (15, 9). The overall rate of HIV patients in the developed, industrialized countries improved drastically in the 1990s, the death rate plummeted and the incidence of various opportunistic HIV-related infections including cryptococcus in the wealthy countries declined significantly. (16,17). However, where most of the world (and even parts of the US) still cannot access this care, the risk of opportunistic HIV-related infection remains huge (18,16,17). Cases of cryptococcosis and death from cryptococcal meningitis are considerably more prevalent in AIDS-related developing countries since the incidence of HIV pandemics is significantly higher and in these countries access to adequate healthcare and therapeutic interventions, including antifungal medicines, is restricted or completely absent (19, 8). Most cases of cryptococcal meningitis occur in countries with low and middle revenues, and about 73% in sub-Saharan Africa. (10) by 2014. Similarly, cryptococcal meningitis in sub-Saharan Africa has and still has the highest mortality rates, mostly due to relatively high untreated HIV/AIDS patients (20,8,10). So, AIDS patients in the US are also faced with a death risk of 15 to 20 per cent, compared with Latin America and Sub-Saharan Africa face 55 to 70 per cent (21,22). As can be seen in developing countries, increased access to HAART may reduce cryptococcal prevalence, fungal meningitis, and associated deaths. Cryptococcosis can also occur in patients with immunocompetence in non-HIV, as monoclonal antiquities, corticosteroids or other immunosuppressant therapies, particularly in countries where HAART has decreased death rates for HIV patients. The use of immunosuppressive treatment regimens is suspected of growing cryptococosis or other fungal infections (23,24). And death rates for pathogens including *Cryptococcus neoformans, C. Albicans* and *A.fumigatus*. Much higher than tuberculosis and malaria (25,26).

After depositing or aerosolizing bird guano, organic matter and soil decomposition, *Cryptococcus neoformans* are a source of atmospheric desiccated cells or spores. (27,28). Such dried cells or fungal spores are inhaled, leading to a first pulmonary infection that often spreads into the brain causing meningoencephalitis (28,29).

Inhaling desiccated, encapsulated or basidiospores triggers human *Cryptococcus* (30). The encapsulated strains are approximately 2-5-micron diameter (31) and can enter alveoli without being expelled through the respiratory epithelium. *Cryptococcus neoformans* in alveolar spaces first face alveolar macrophages (32), which play a key role in *Cryptococcus neoformers* defence. Stimulated cells can bind, ingest and kill macrophage (33,34). Phagocytosis can occur via antibodies (35), supplemental receptors (36) β-glucan (33) and mannose (37). Primitive opsonins or collectins contribute to innate resistance to inhaled microorganisms in mammals and birds in Alveoli (37,39). They belong to the C-type lectin superfamily, defined by Carbohydrogen Recognition (CRD) ligands with Ca2+ collagen tail (40). Resistance and use of numerous hypoxia and tension enzymes (41,42). It also produces a range of metabolites that provide survival benefits and establish an essential micro-environmental fungal-like mannitol, trehalose, ethanol and acetate (43,44,45). Cryptococcal disease infection occurs for any area of the body, including lung, spleen, prostate, skin, brain, liver, lymph nodes and bone (46). Of all cryptococcal infection sites, the most commonly affected are pulmonary and CNS sites with most CNS mortality (47).

Cryptococcus Ecological Niche

Cryptococcus neoformans are present outside the human host in very different ecologically diverse niches, depending on the local climate. In 1894, Sanfelice isolated the first strain of *Cryptococcus neoformans* from fruit juice (48). Moreover, the lack of ecological environments can affect *Cryptococcus neoformans* distribution and virulence in geographical areas. (49,50). Pigeons have only a latent infection, not an active infection, as their higher body temperatures are not ideal for fungal growth. But they are good vectors because their excrement provides nutrients to survive (51,52). Moreover, because *Cryptococcus neoformans* can live in saprophytic shape, they are present in all surfaces, including polluted soil, fruit and vegetables that come into contact with bird dropping, including in houses where birds can enter through open windows. This is especially dangerous for HIV-positive patients in these areas, as they are often re-infected at home (53,49). *Cryptococcus* species can live in and reproduce in soil nematodes and freely-living amebae (54). However, the prevalence of *Cryptococcus neoformans* in terms of host tree species is more prevalent than *Cryptococcus gatti* (17) *Cryptococcus neoformans* host tree species versus 12 *Cryptococci gatti* (49), which results in a wide distribution of the arbours for *C.neoformans* and thus an increased probability of the interaction of the host-pathogen. In 2003, Malik submitted a study of cryptococcosis of Australian parrots and reported that *Cryptococcosis* in parrots includes the nasal

cavity and upper respiratory tracts, beak, sinuses and face surfaces. It seems to have been a predisposing factor for *Cryptococcosis* to sit parrots on the eucalyptus trees that contain *Cryptococcus neoformans* yeast. (55) Seo published a prostatitis study with *Cryptococcus neoformans* in 2006. In an immune-deficient alcoholic patient having cirrhosis, he registered prostatitis with *Cryptococcus neoformans*, with a diagnosis of sonography and biopsy (56) In 1993, Li isolated *Cryptococcus neoformans* from pigeon faeces in China and reported that 78% of A serotypes and 22% of AD serotypes. It found only *C.neoformans* variety from pigeon faeces in China, although the *Cryptococcus neoformans gatti* variety was also separated from clinical samples in China and has a special nature and is unique to tropical and subtropical areas (57). Duncan (2006) obtained *Cryptococcus gattii* from grey squirrel cultivation in Vancouver Canada and reported that wild animals of Vancouver, like domestic animals in this area, can be a reservoir for this fungus (58).

Mating

The life cycle of *Cryptococcus neoformans* (**Fig.1**). Under nutrient-limiting conditions,the peptide pheromones that cause cell-cell fusion are secreted by a and α yeast cells. The resulting dikaryon is causing filamentous growth and the two parental nuclei migrate in hyphae in coordination. To separate the cells, a septum forms, a nucleus is transferred via a clamp connection to the penultimate hyphal cell, and the clamp cell and hyphal cell fuse. Blastospores (yeast-like cells) may bud from the hyphae during this hyphal growth and divide in the form of the yeast mitotically. Chlamydospores can be enlarged and formed by some hyphal cells. At the basidium formation stage, the two nuclei fuse and undergo meiosis to create four meiotic products that form basidiospore chains via mitosis or surface budding. Diploid **α/α** cells, during monokaryotic fruition, for example, become **α/α** cells either by endoduplication or nuclear fusion following cell fusion between two α cells. Rudimentary clamp connections form the diploid monokaryotic hyphae, but these are not fused to the preceding cell. During fruiting, as in mating, blastospores and chlamydospores also form. Meiosis occurs at the stage of basidium development and haploid basidiospores in four chains are produced (59).

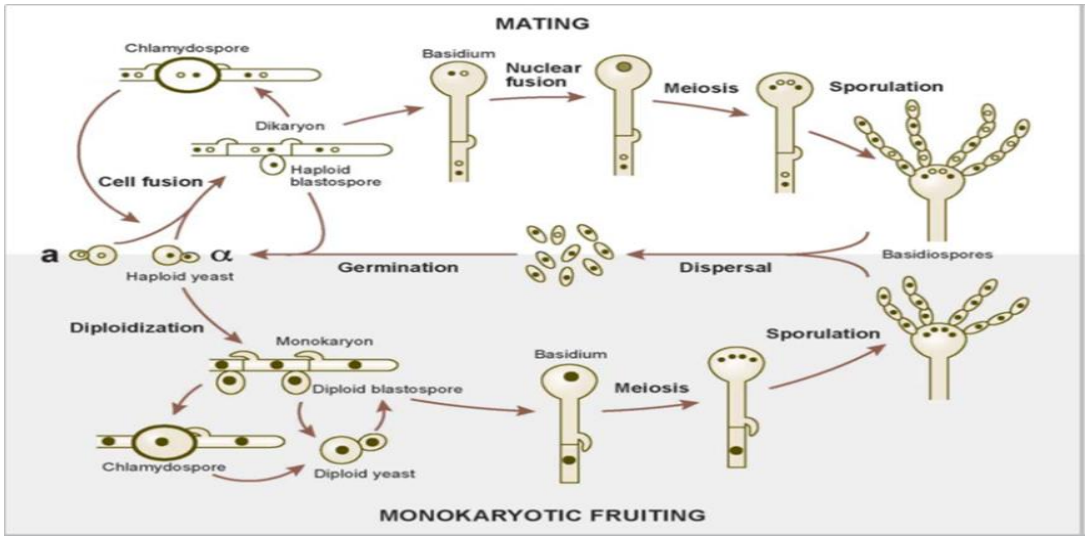

Fig.1. The life cycle of *Cryptococcus neoformans*

Infection process and host response

Cryptococcus neoformans can enter the respiratory system in the human host by inhaling spores or airborne yeast cells from the atmosphere (60). The encapsulated fungal cells are frequently approximately 5 to 10 μm and are thus prone to a mucociliary clearance from lung epithelia (61). *C.neoformans* basidiospores and dedicated cells, however, measure around.6-3 μm, sufficiently small for alveolar deposition following inhalation (36), probably from the mucosal movement itself (29), isolated from the soil or bird droppings. *Cryptococcus* cells can survived out of the cell and/or transmission into the pulmonary cell once in the alveolar sphere either through direct internalization through resident alveolar macrophages or the pulmonary epithelial cells (62,63). In this stage pulmonary colonization, cryptococci are either cleared or become a localized latent asymptomatic infection, is triggered depending upon the existence of the host immune response. Lungs involvement may also be a temporary stop for cryptococci to develop symptomatic infection and ultimately spread to other parts of the body. Alveolar macrophages constitute 95 per cent of the broncho-alveolar cells, making them the predominant pulmonary phagocytes in the lung (64). Cryptococci relationship with alveolar macrophages is probability to decide the establishment and fate of pulmonary infection and possible systemic spread. *Cryptococcus neoformans* ability to stay and grow within macrophages could explain immunocompetent hosts' latent cryptococcosis. Studies using the rats *Cryptococcus* model found that immunocompetent strongly resemble rats. Developing granulomas and cryptococcal infection pulmonary containment distinguishes this model. This reverse reaction is reversed by treatment murine with immunosuppressed dexamethasone, leading to loss of granuloma formation and increased lung

fungal burden (63). The two less dangerous outcomes are that the host's immune system can handle and purge the spores, or the infection can remain latent and lung-free. (29). The third finding is that latent infection, such as HIV or pharmacological immune suppression, can revive the lung after the immune system has weakened, leading to more extreme outcomes (64) and lung inflammation and lung disease-causing. Cryptococcal spread from pneumonia to other tissues, such as the urinary, prostate, eyes, bones, liver, spleen, lymph nodes, and in particular, the brain is the fourth and most harmful outcome (46). The organ that is most frequently affected by host-infected *Cryptococcus neoformans* is lung and brain, and not just lung infection in people affected by the disease may cause pneumonia, but blood infection also leads to fatal meningoencephalitis (29). In immunocompetent individuals, there is a range of phases in the immune response to infection. When *Cryptococcus neoformans* cells enter the alveoli in the lungs, alveolar macrophages attempt to phagocyte and either kill or sequestrate fungal cells into granulomas (65,18). As the engulfing macrophage succeeds, the fungal cells are secreted within the phagolysosome, a phagosomal organelle formed by a lysosome fusion that creates a local environment where low pH, hydrolytic enzymes, anti-microbial peptides and free toxic radicals are produced. (66,67). Specifically, free radical species such as RS and RNS can damage the cell wall and cell membrane and attack DNA and cell proteins (68,69) while pathogenic proteins are divided into peptides in hydrolytic enzymes. The pathogens have been destroyed and their peptides is shown to release cytokines which attract neutrophils and other immune cells through a major histocompatibility complex (MHC) cell surface receptor T-cells and macrophages (70).

Cryptococcal serotypes and genomes

Capsular agglutination reactions have identified five cryptococcal serotypes and are further categorized in nine molecular groups based on polymorphisms of DNA sequence. *C. neoformans* var. *neoformans* are composed of serotypes D (molecular type: VNIV) and AD (molecular type: VNIII) and C hybrid serotype *C. neoformans* var. *grubii* is made up of serotype A (molecular types: VNI, VNII, VNB) and *C. gattii* consist of B (molecular types: VGI, VGII, VGIII) and C (molecular: VGIII, VGIV), (71,72,73) serotypes (Fig.2). All, molecular and phylogenetic studies attribute the development of two distinct monophyletic lines for C to reproductive isolation. *C. neoformans* and *gattii* (74,75). Genetic and molecular studies based now on the genome sequences available provide the means to research the role of particular genes in virulence *C. neoformans.* Sequencing of genomes for *C.neoformans* has been completed. JEC21 and B-3501A serotype D strains and serotype A strain H99 (the most common serotype

comprising more than 95 per cent of *Cryptococcal* infections in AIDS patients), along with *C.gattii* strains. WM276 strain of serotype B and R265 clinical strain. The genome of strain JEC21 is as a representative example, composed of 14 chromosomes, totaling 20 Mb of DNA, and a projected 6,574 genes (76).

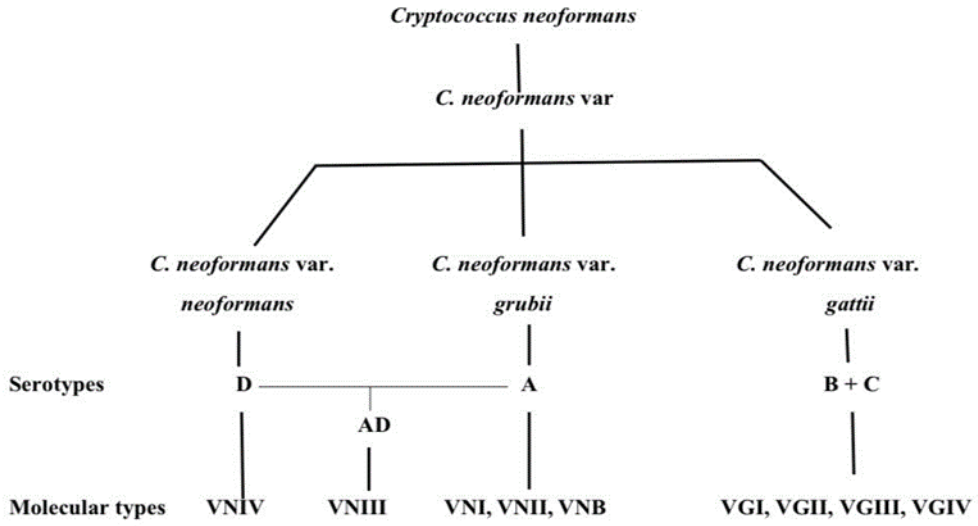

Fig.2. Serotypes and Molecular Types in *C.neoformans*

Factors of virulence

The pathogens live in a complex relationship to the infected host. The pathogen needs to feel the host environment during the initial encounter and respond to adaptive cellular changes. The response also includes inducing specific phenotypes that enhance the capacity of the microorganism to survive and develop in this new environment (77). *Cryptococcus neoformans* fungus is relatively common cause of life-threatening meningoencephalitis in patients with compromised immune systems or in patients with serious immune defects (78). *Cryptococcus neoformans* have several known factors for virulence including their capacity to grow at a temperature of 37°C, polysaccharide capsules and their ability to produce suitable melanins. Virulence also includes various proteases, lipases and other enzymes, as well as several metabolites generated by *Cryptococcus neoformans* after infection (79).

The Capsule

The capsule is the prevailing virulence factor in *Cryptococcus neoformans* and plays a major role in this fungus' biology. The capsule defends fungi from phagocytic predators and field desiccation. The capsule interferes with the

immune response and provides a defensive shield for the fungal cell that is antiphagocytic and capable of consuming phagocytic cell-borne microbicidal oxidative explosions(80). The capsule consists mainly of polysaccharides and includes two major polysaccharides: glucuronoxylomanane (GXM) and galactoxylomanane (GalXM). GXM weights 90–95 percent and GalXM about 5–8 percent. A small percentage (<1%) of mannoproteins (MPs) was also identified(82). The fungal spores are normally non-encapsulated during inhalation (28), as smaller in size allows the airway through, but during infection, it increases dramatically as the spore enters the alveoli. If there is phagocytosis, the polysaccharides in capsules are released into the vesicles macrophages around the phagosomes (or phagolysosomes), and the build-up of these vesicles in the host cell cytoplasm leads to macrophage and lysis. The capsule is also used to fight macrophage attempts to kill fungal cells macrophages that invade cryptococci (82). Shortly after infection, the capsule increases dramatically in size. In vitro the capsule can grow as fast as 0,3-2,5 um^3/min and appears to have an effect on its final size (83). Capsulation size and composition reflect extracellular factors. In-vitro capsule expansion requirements include low iron, mammalian serum, high CO2, mannitol, and nutrient appetite. Tiny capsules with high osmotic pressure, nutrients and iron are observed (84,85).

Genes associated with capsules (CAP genes)

There are four genes (*CAP10, CAP59, CAP60* and *CAP64*) associated with the *Cryptococcus neoformans* polysaccharide capsule. (86,87). *CAP59* has primarily been found as an important gene for the development of capsules and virulence in mice and is assigned to Ch. I (88). Protein Cap59 was known as a transmembrane protein (89). The second capsule-associated gene for chromosome III was *CAP64* (87). The *CAP64* gene supplemented an a capsular strain 602, which losing the capacity to manufacture capsules, for producing the capsule and causing fatal infection in the murine, while strain 602 was virulent (90). *CAP60* is the third capsule-linked gene of Ch.I and Cap60 protein across the nuclear membrane. (91). The *CAP10* gene was identified on various chromosomes compared with the other three capsule genes, and the cytoplasm of the protein encoded by the *CAP10* gene. The complement of the CAP10 gene-deficient a capsular mutant formed an encapsulated strain and of *CAP10*Δ from the wild strain triggered the production of a phenotype-like an capsular (86). In capsule synthesis, all four CAP genes were stated to be important, but Biochemical properties of CAP gene products have still not been identified.

Production of melanin and lacceas activity

Another key virulence factor is melanin production. Melanin protects against ultra-violet (UV) environmental radiation, provides the cell wall with structural support and protects against phagocytosis, and macrophage-related oxidative killing, and contributes to extrapulmonary dissemination (92,93,94). melanin or melanin-like pigments are manufactured using copper as co-factor by laccases, which are members of the protein multicopper oxidase family (95). Since laccase-related mutants, these laccase enzymes are important. Virulence reduction in *C. neoformans* with the corresponding melanin deficiency (93). Melanin is produced in *Cryptococcus neoformans* using two lacca enzymes, Lac1 and Lac2. Lac1 is closely linked to the cell wall, while Lac2 is present in the cytoplasm (96). Once melanin is formed, it is deposited into cells forming a dense electron layer of (97) where melanin has its antioxidant function, protecting the cell walls, membranes and other internal parts of cryptococcal cells from free oxygen and nitrogen radicals as well as other macrophagic toxic molecules (98,99). Fungal cells can be neutralized and shielded from the antimicrobial oxidative effect of hydroxyl radicals in macrophages from Fe(II) laccase enzyme to Fe(III)(100,101).

Thermotolerance

C. neoformans ability growing and surviving at 37°C significantly contributes to their function as a human pathogen. One of the first problems faced by the fungus when joining the human host is the temperature rise. The thermotolerance of *C.neoformans* shown was due to pathway signals. (102,103). Also, *C. neoformans* has developed two main temperature rise resistance mechanisms. Firstly, the prevention of protein denaturation and the ability to restructure proteins using trehalose disaccharide and heat shock protein chaperones.

The second mechanism involves the use of superoxide dismutase to protect antioxidants (104,105). The mitochondrial superoxide dismutase (Sod2), a major component of the antioxidant defence mechanism in *C.neoformans* is in particular. are also linked to growth adaptation at high temperatures (104). This virulence is present in less than 0.01% of outdoor fungi and is absent in most soil fungi and most cryptococcal species (27,106).

Acetate

The pathogen generates a variety of metabolites, including acetate, that gives survival benefit by the formation of an optimum micro-environment (45,107). Acetate is one of the main in vitro-cultivated cryptococci metabolites (43).

Acetate was also found to be a significant infection-related metabolite based on brain and lung tissue biopsy studies of infected rats (108). Moreover, significant quantities of acetate were detected by nuclear magnetic resonance (NMR) from pulmonary cryptococcosis (109). While acetate is not fully explained role in virulence, it has been shown to increase fungal survival, perhaps via immunomodulatory mechanisms (45,110).

The Xfp1/2 – Ack pathway which produces acetate from D-fructose 6-phosphate or D-Xylulose 5-phosphate and the Pdc-Ald pathway for producing pyruvate acetate have established two possible pathways for the production of acets in *Cryptococcus*. Acetate, which can be converted to acetyl-CoA in the tricarboxylic acid cycle, gluconeogenesis, or glyoxalate cycle, is one of *Cryptococcus neoformans* carbon sources. It was also shown that *Cryptococcus neoformans* produce high in vitro acetate concentrations and lung tissue infection in the mouse model. (45). Acetate is thought to provide the pathogen with, among other ways, a survival advantage due to its effect on pH. *Cryptococcus neoformans* grow only within a certain pH range, unlike other fungi, such as *Candida albicans* (*Aspergillus fumigatus*), but this range has a pH of 7.4, which is the pH of human blood, brain fluid and acidity of the macrophage phagol. When you grow outside this preferred host body acid range, such as cerebral cryptococomas, the pathogen secrets the tissues with excess acetate to reduce local pH (44). This optimizes the function of phospholipase B and other cryptococcal enzymes (111,112). The decreased pH in the environment around *Cryptococcus neoformans* would protect the pathogen from immune attack by decreasing or increasing neutrophil neutralization, allowing free radicals to neutralize and decrease superoxide production, reducing immune cells' ability to use certain chemical agents to kill infected cryptococci (45) .

Phospholipase

Phospholipases are a heterogeneous community of enzymes which can hydrolyze glycerophospholipid ester connections. The enzyme of *Cryptococcus neoformans* has lysophospholipase hydrolase, PLB and lysophospholipase activity of transacetylase (113). Phospholipase activity may trigger membranes to become destabilized, cell lysis and the release of secondary lipid messengers, interstitial pulmonary infection, and the spread of *Cryptococci* in both lymph and blood (114,115). Phospholipase B is a key component of lung surfactant dipalmitoyl phosphatidylcholine that increases the bond with lung epithelial cell, thereby assisting fungal spread (115,119,27). Macrophage arachidonic acid, then used for the generation of eicosanoids (117,118), is taken by cryptococci. The

developed eicosanoids can be used to suppress the immune response of the host to promote intracellular survival and fungal propagation (117,119).

Proteinase

These proteinases and phospholipases have been further proposed to allow *Cryptococcus neoformans* to be replicated within the host Macrophages by harming phagosomal membranes and thus avoiding the killing of phagocytic enzymes. Despite this advance years ago, no additional work was done to elucidate the mechanism used to increase *Cryptococcus neoformans* virulence in a host by proteinases (120).

Mechanisms of dissemination

The BBB (blood brain barrier) ensures that the brain is strongly secured and that macromolecules and microorganisms circulate with little access. The human BBB consists of microvascular, astrocyte, pericytes and neuronal feet supported endothelial cells (121,122). Unlike peripheral endothelial cells, close junctions bind brain endothelial cells, rendering the blood brain barrier a great barrier to many pathogens (122,123). *Cryptococcus neoformans* must cross the blood brain barrier(BBB) that is normally impermeable to infect the brain. It is currently clear that cryptococcal yeast cells will use a variety of ways to enter the brain once in the body. *C. neoformans* have shown a preference for infecting CNS (central nervous system) by several factors including the existence of neuronal substrates for fungal growth, a refuge place for host immune response, fungal survival and proliferation capabilities in hypoxic environments, and the ability to attract fungal cells by a neuronal cell receptor (124,12). Following an effective breach of the CNS, *Cryptococcus neoformans* can cause diseases especially meninges and brain infection and inflammation (**Fig.3**).

Model of Trojan dissemination of horse

Cryptococci live and proliferate within macrophages following phagocytosis (125,126). In addition, in a novel non-lytic exocytosis (monocytosis), Live *Cryptococci* may be removed, leaving the macrophage unharmed. The exocytes then migrate to other cells (125,126,127). This spreads to other cell can possibly explain the use of phagocytes in cryptococcal cells to penetrate the blood brain barrier by hitchhiking through host phagocytes (Trojan-horse ways) (128).

Transcellular pathway

Cryptococcus neoformans is used to reshape the endothelial Cryptococcal protein kinase-dependent actin, using hyaluronic acid on its surface to hook it up

to CD44 in luminary endothelium. Then the fungal cells leave the other side and therefore cross the blood brain barrier through the endothelial cells (129,130).

Paracellular pathway

The pathway contains pathogens breaching the intercellular blood brain barrier (131,129). This process involves the degradation and weakening of pathogenic, close junctions linking brain endothelial cells, Chen et al. showed that microvascular endothelium cryptococcal binding induced close junction alteration (132).

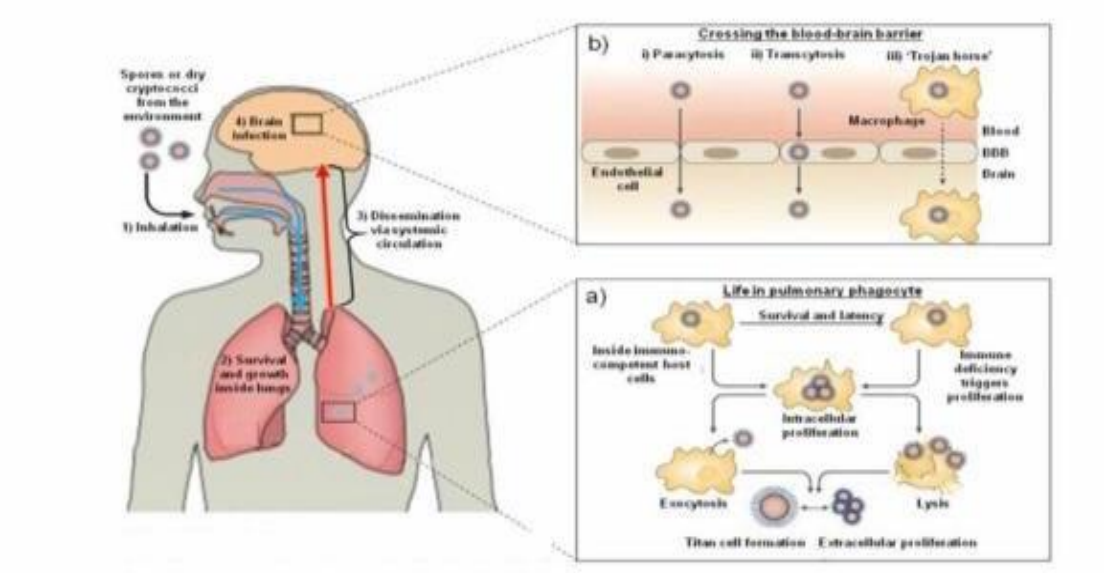

Fig.3. Mechanisms of dissemination *C.neoformans* in BBB.

Ubiquitin

The degradation of a protein is not a simple process biologically. Peptide bonds in physiological environments are very stable and are an obviously beneficial feature, since a cell with spontaneously degraded proteins is difficult to imagine. However, it is often essential that proteins are destroyed. It also has to remove and recycle damaged proteins (133). In this case it is not just necessary to destroy the correct protein but at the right time. The protein system developed by the cell is a ubiquitin (Ub) (76 amino acid polypeptide) that was produced in the cell to label destructive tag proteins. This ubiquitin(Ub) tag is used as the signal for the proteasome to degrade the protein. One of the main pathways for intracellular proteolysis is this ubiquitin- proteasome pathway. Ubiquitylation requires an isopeptide linkage between ubiquitin and a lysine side chain on the substrate (134). On one site a single ubiquitin can be conjugated (monoubiquitination) or multiple Ub can bind to shorter oligoubiquitin chains through 1 of the 7 lys residues of Ub (2- 4 Ub) or longer chains of polyubiquitin

(4-Ub) (135). Ubiquitin is activated first with its residue from C-terminal glycine. This is achieved by E1(ubiquitin-activating enzyme), the enzyme uses adenosine triphosphates to make ubiquitin adenylate and is used as a substrate for the synthesize of ubiquitin thiol ester (136). After that E1 is then trans for E2 (ubiquitin conjugating enzyme) then end to the substrate by E3 (ubiquitin ligase) (137). The E3 ligase which forms a ubiquitin thiol ester, which can transfer Ub to the substrate, or indirectly convert the substrate into a platform for E2 and an interacting substrate, depending on its sort (138). The existence of a polyubiquitine chain targets proteasome substrates that use adenosine triphosphates energy (139). Then the substrate is analysis into ubiquitin and oligopeptides, released by deubiquitylation enzymes from the substrate, can then be recycled (140). only a small number existed ubiquitin-activating enzyme and ubiquitin conjugating enzyme, hundreds of known E3 ligases still have to be discovered. The 2 largest classes of ubiquitin ligase(E3) ligase are distinct in their ubiquitin molecule transfer mechanism and sequence: they are the new gene (RING) (138) and the new E6-AP carboxy terminal are of very interest (HECT)(141). RING are proteins bringing the ubiquitin conjugating enzyme, target protein and moving ubiquitin from one to the other. HECT function as mediates, the ubiquitin is first transferred from the E2 to itself and then converted into the target protein and this in RING E3 ligases not found.

F-box protein

F-box protein(FBP) a pattern of around fifty amino acids that function as a site of interaction between protein and protein (142). The hypothesis says FBP work as scavenger elements in cell that gathers proteins to be sent to the SCF complex. Ubiquitin is marked for the junk proteins in the S26 (proteasome) in SCF complex (143,144). The theory of the F-box protein is founded on the idea that an F-box mediates structure into a SCF by connecting it to the Skp1. The SCF complex (Fig.4) compose of: F-box protein (FBP) (143), Skp1 (Kinetochore protein mutant suppressor) (145), Rbx1 (ring-box protein) also known as Hrt1 or Roc1 (146) and Cul1 (Cullin) (147). Since Fbps act as the factor for the identification of the substrates of the SCF ligases, many Fbps ensure high substrate specificity (148). Cullin 1, RBX1 and SKP1 weigh respectively 89,7, 12,3 and 18,7 kDa , while the mass of the F-box protein ranges from 47 to more than 110 kDa (149) . The F-box is normally in the amino-terminal half of the protein and is mostly coupled in the carboxy-terminal of the protein, two of which are most typically leucine-rich repetitions in humans (LRRs) and WD repetitions. The human F-box protein nomenclature proposed by the Human Genome Organization fits the trend proposed by Cenciarelli (1999) and Winston (1999): FBXL is a protein that includes F-box

and LRRs; FBXW is a protein that includes F-box and WD, and FBXO denotes an F-box protein and either another or no other pattern (150,151).The fungal F-Box proteins are essential to cell functions such as circadian clocks, transcriptions, development, signals transduction, cell cycles and sensing of nutrients (152). The FBP does not function randomly, but provide certain proteins which are frequently changed in SCF complex and thus regulate protein levels in a cell (143). SCF complexes promote the interaction between substrates and enzymes, which then transfer ubiquitin to substrates. The 26S proteasome subsequently degrades poly-ubiquitine substrates. The FBP is the subunit of the SCF complex that connects certain substrates to the complex and connects it to the complex through the F-box itself. There are numerous SCF complexes in both yeast and human cells that only differ in the F-box protein ingredients. Three characteristic SCF complexes are available in yeast: SCFMet30, SCFGrr and SCFCdc4, designated for their F-box portion (153). When phosphorylated, F-box protein targets are identified. Such phosphorylation can be carried out by various protein kinases such as Pho kinases, CDK's, CK's and MAPK's according to the way the target protein works (154). Losing a fungal protein from F-Box is sometimes pleiotropic, particularly in cases where the F-Box has many objectives, the null mutation is lethal for Cdc4, which has 10 identified targets. Conversely, if the deletion of the gene is of little or no consequence, the FBP can only target one or a few proteins (154). The amino-terminus of Cullin1 linked with FBP through SKP1 (155). Cullin1 is composed form amino-terminus helical region and a carboxyl- terminus globular α/β domain (156).

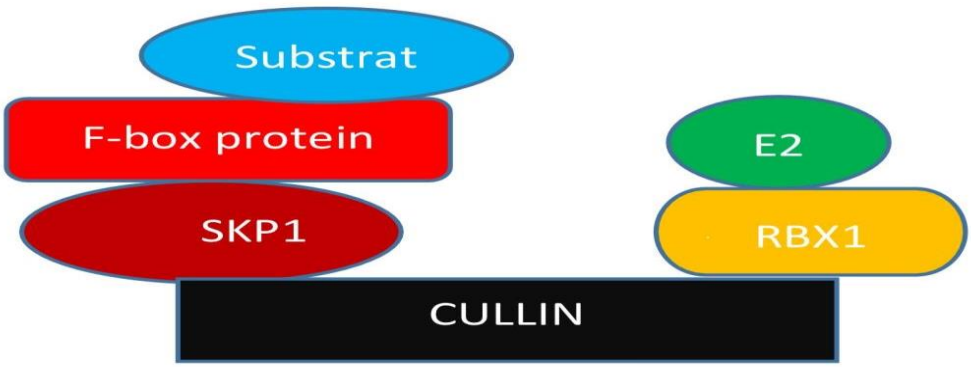

Fig.4. Schematic view of the SCF complex

F-box protein in disease

In various pathways of biological development, F-box proteins regulate substrates which control key dimensions of cell life, including cell division, cell growth, development and differentiation, signaling , and cell survival and death.

Therefore, F-box protein ubiquityulation dysregulation that can occur via several distinct mechanisms (157). The F-box protein Fbp1 is important for fungal plant pathogen invasive growth and virulence *Fusarium oxysporum*. The *fbp1*Δ is also hypersensitive to white calcofluorine and sodium dodecylsulfate resulting in decreased cell wall phosphorylation. These findings indicate that Fbp1 contributes both to *Fusarium oxysporum* invasion and to the integrity of the cell walls (158). F-box protein Fbp1, which causes little damage in the infected lung, is important regardless of classic virulence factors (capsule, melanin), *fbp1*Δ cannot spread to other organs in the mouse model after a pulmonary infection. but still contributes to a brain infection in the model of intravenous murine injection that shows that the *fbp1*Δ is unable to leave from the pulmonary system (159). Fbp1 is important for *Cryptococcus neoformans* fungal sporulation and virulence. Fbp1 was identified as important for fungal virulence as *fbp1*Δ in of mouse systemic infections were a virulent. Basidiospore development in bilateral mating between *fbp1*Δ was blocked, despite the presence of normal dikaryotic hyphae during mating (160). That FBP1 in *G. zeae* is important for multiple phenotypes including both virulence and sexual development (161). The dimorphic *Candida albicans* switch between the yeast form, pseudohiphal form and the true hyphal type is central to the invasion and development of the host disease and an essential virulence characteristic. Two Grr1 and Cdc4 for f-box proteins are listed as essential in this morphological. Either removing GRR1 or CDC4 from the genome of the *Candida albicans* results in pseudohyphal or filamented morphology, under conditions which generally contribute to yeast growth (162,163). The repression of pseudo-hyphal production from Grr1 may be caused by the negative cytokinesis control by two G1 cyclines, Cln3 and Ccn1, which is similar to the *Saccharomyces cerevisiae* regulation. These cycline proteins are stabilised in a *grr1Δ* that prevents cell division after cytokinesis, which suggest that they are potential Grr1 substrates. Furthermore, the Hof1 cellular level of the *grr1Δ* cell is also increased significantly, a protein that plays a role in cytokinesis (164). In the meantime, the way Cdc4 controls cell morphology is less evident. One Cdc4 substrate, Sol1, known to play a part in *Candida albicans* morphology (165). The most destructive rice disease is *Magnaporthe oryzae*, study identified that FBP, Pth1, which is important for both rice and barley fungal disease. Pth1 is Grr1 homologue is required to regulate appressorium maturation, a specialist cell structure for host cell penetration. The *pth1Δ* does not the host leaf surface, establishing a strong host-pathogen relationship, research showed that Pth1 is necessary for the metabolism of fungal carbohydrate and the generation of hydrostatic pressure in appressoria, which can lead to defect appressoria in *pth1Δ* (162). FBPs (SKP2, FBXW7, and β-TrCP) research focused on cancer. SKP2 facilitates S-phase

entering by striving for proteasomal-dependent degradation with the CDK inhibitor p27. This role makes SKP2 an oncogenic FBP epitome. SKP2 over-expression is related a variety of cancers, this function was confirmed through studies in mouse models. The SKP2 inactivation induces cell senescence independent of p53 and prevents tumorigenesis (166,167).

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