4 Pathogenesis of COVID-Associated Mucormycosis

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4.1 Introduction

Mucormycosis is a serious fungal infection caused by ubiquitous fungi which belong to the order Mucorales. The common genera infecting humans include *Rhizopus* spp. (most commonly *R. arrhizus, R. microsporus*, and *R. homothallicus*), *Rhizomucor* spp., *Mucor* spp., *Cunninghamella* spp., *Lichtheimia* spp., and *Apophysomyces* spp. [1]. The major underlying health conditions predisposing to mucormycosis include diabetes mellitus (DM) and immunosuppression; trauma such as burns or road traffic accidents, and iron chelation therapy with deferoxamine [1–3]. An upsurge in the cases of mucormycosis has been noted over the last decade in the developed and developing world alike but has been especially concerning in Asia and specifically in India. Recently, a dramatic rise in the cases of mucormycosis infection has been observed in patients with Coronavirus-19 disease (COVID-19) or those recovering from it, as evidenced by several reports and institutional experiences [1, 3, 4]. COVID-19 is a viral inflammatory disease caused by SARS-CoV-2 characterized by an exaggerated proinflammatory response with cytokine surge, altered iron metabolism and iron overload, hemoglobinopathy, hypercoagulability state causing multisystem involvement [5–8]. Several strategies to prevent and treat mucormycosis have been used over the years, and more such strategies are urgently needed in view of its raging rise [9]. It is essential to have a clear understanding of the pathogenesis of this disease to facilitate these efforts and ensure appropriate management. Therefore, in this chapter, we will provide an overview of the pathogenesis of mucormycosis, focusing on rhino-orbital-cerebral mucormycosis (ROCM) in patients with COVID-19.

4.2 Host Risk Factors

Mucormycosis generally occurs in immunocompromised individuals with defined risk factors. Table 4.1 shows the risk factors and underlying diseases associated with mucormycosis. Diabetes mellitus is the most common underlying disease, followed by hematological malignancies and solid organ transplants [17]. Hyperglycemia is associated with defective neutrophil migration, chemotaxis, and phagocytosis [18, 19]. Other predisposing factors associated with mucormycosis are patients receiving iron chelation therapy and steroids. In India, 3–26% of mucormycosis cases were recorded from the immunocompetent host, compared to 18–19% globally and they usually present with cutaneous or isolated renal mucormycosis [17]. Trauma is the most common

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Table 4.1 Risk factors for mucormycosis

Predisposing condition	The predominant site of infection	Most common species	Reference
Diabetes, hyperglycemia, and ketoacidosis	ROCM	*Rhizopus* species *(R. arrhizus, R. microsporus, R. homothallicus)*	[10]
Hematological and solid organ malignancy	Pulmonary, ROCM	*Rhizopus* species, *Cunninghamella* species, *Lichtheimia* species., *Cokeromyces* species	[11]
Solid-organ transplant, HSCT	Pulmonary, ROCM	*Rhizopus* species, *Cunninghamella* species, *Lichtheimia* species	[11]
Neutropenia	Pulmonary, disseminated	*Rhizopus* species, *Lichtheimia* species. *Cunninghamella* species	[11]
Iron overload and iron chelation therapy	Pulmonary, disseminated	*Rhizopus* species, *Lichtheimia* species, *Cunninghamella* species	[1]
Corticosteroids	ROCM, pulmonary, disseminated	*Rhizopus* species, *Cunninghamella* species, *Lichtheimia* species	[1]
Malnutrition, preterm neonates, neonates with suspected necrotizing enterocolitis	Gastrointestinal	*Rhizopus* species, *Lichtheimia* species	[12]
Breach of skin (trauma due to accidents, burns, catheterization, injection site)	Cutaneous	*Apophysomyces* and *Saksenaea* species *Syncephalastrum* species	[13]
New risk factors:			
SARS-CoV-2, long term steroid use for the treatment	ROCM, pulmonary	*Rhizopus* species *(R. arrhizus, R. microsporus, R. homothallicus), Lichtheimia* species	[14]
Post pulmonary tuberculosis/ COPD	Pulmonary mucormycosis	*Rhizopus* species	[15]
Breakthrough mucormycosis after voriconazole treatment	ROCM, pulmonary	*Rhizopus* species	[16]
Immunocompetent host in the Indian subcontinent	Renal mucormycosis	*Rhizopus* and *Apophysomyces* species	[1]

ROCM Rhino-orbito-cerebral mucormycosis, *SARS-CoV-2* severe acute respiratory syndrome coronavirus 2, *COPD* chronic obstructive pulmonary disease, *HSCT* Hematopoietic Stem Cell Transplantation

risk factor associated with cutaneous mucormycosis [11]. Newer risk-factor like SARS-CoV-2 is emerging. The virus itself acts as a risk factor by decreasing immunity and irrational use of steroids for the treatment, making individuals susceptible to the infection [14].

4.3 Virulence Factors of Mucorales

Virulence factors are an essential part of Mucorales that allow these species to invade and survive within the host tissue during infection. The critical virulence traits essential to establish disease are summarized in Table 4.2.

4.4 Pathogen Entry

The agents of mucormycosis can gain entry to host tissues via various modes. The primary barriers against an invasion of any external pathogen include nasal mucosa, sinus cavities, skin, and endothelium layers. Typically, Mucorales are incapable of penetrating intact skin. Still, any breach in mucosal continuity by trauma or injury can promote fungal adherence to components of the basal epithelial cell layer such as laminin and type IV collagen [28]. Inhalation of Mucorales sporangiospores is the main entry portal. While the development of mucormycosis is not seen in immunocompetent hosts, those with risk factors can develop a progressive infection at various

Table 4.2 Virulence factors for Mucorales

Virulence traits	Function	Mechanism of virulence by Mucorales	Reference
Iron metabolism-related virulence traits *Reductase/permease systems*			
Low-affinity iron reductase	Performs in iron-rich environments and reduces iron	Patients with DKA (pH -7.3–6.88) reduce ferric ions to ferrous and facilitate *Rhizopus* spp. growth	[20]
High-affinity ferric reductase	Functions in iron-depleted environments and reduces iron	In DKA, carrier molecules bind to iron, and free iron is available only in submicromolar concentrations. The high-affinity ferric reductase facilitates the uptake of bound iron by *Rhizopus*	[20]
Other ferric reductases	Reduce iron	Allow iron uptake in iron-depleted environments and facilitates *Rhizopus* spp. growth	[21]
Copper oxidase (Cu-oxidase)	Oxidize iron	Trans cell membrane transport of iron	[20]
High-affinity iron permease (FTR1)	Helps in the transport of iron	Trans cell membrane transport of iron	[22]
Role of siderophores in Mucormycetes iron metabolism			
Siderophore permeases	Provide iron to cells by chelating iron	This siderophore supplies iron by direct transfer across the plasma membrane for *Rhizopus spp.* growth	[23]
Rhizoferrin	Siderophore that chelates iron	This siderophore supplies iron by the energy-dependent process for *Rhizopus* spp. growth	[24]
Deferoxamine	Deferoxamine extracts ferric iron from transferrin	It acts as a xenosiderophore. It attaches on the *Rhizopus* spp. and functions by transporting iron by energy-dependent reduction of iron	[25]
Role of hemin utilization in Mucormycetes iron metabolism			
Heme oxygenase	Iron uptake from heme	Obtain iron from host hemoglobin and allow angioinvasion of *Rhizopus* spp.	[22]
Others			
Proteinases • Aspartic proteinases • Subtilase protein	Protein lysis	Degrade host epithelium and contribute to the angioinvasive nature of the disease	[26]
Rhizoxin	Mycotoxin	Antimitotic metabolite that kills host cells	[27]

GRP glucose receptor protein

sites (Fig. 4.1). In patients with burns or other skin trauma/maceration, the spores can directly penetrate deeper tissues. Spores can also gain entry via application of non-sterile adhesive tapes and surgical dressings [29, 30]. Furthermore, mucormycosis spores can also gain access via contaminated tongue depressors or wooden applicators [31].

4.4.1 Spore Germination

The germination of spores and hyphal formation is critical for establishing infection in the host. In immunocompetent mice, pulmonary alveolar macrophages harvested from the lungs have been found to effectively ingest and prevent the germination of *R. arrhizus* spores both *in vitro* and after intranasal infection. In contrast, those derived from immunosuppressed mice are unable to do so [32].

4.4.2 Attachment and Invasion

The next step is attaching to extracellular protein matrices such as laminin and collagen IV. Any epithelial cell damage (e.g., due to COVID-19) can expose them for interaction with inhaled/ingested spores [28]. *Rhizopus*

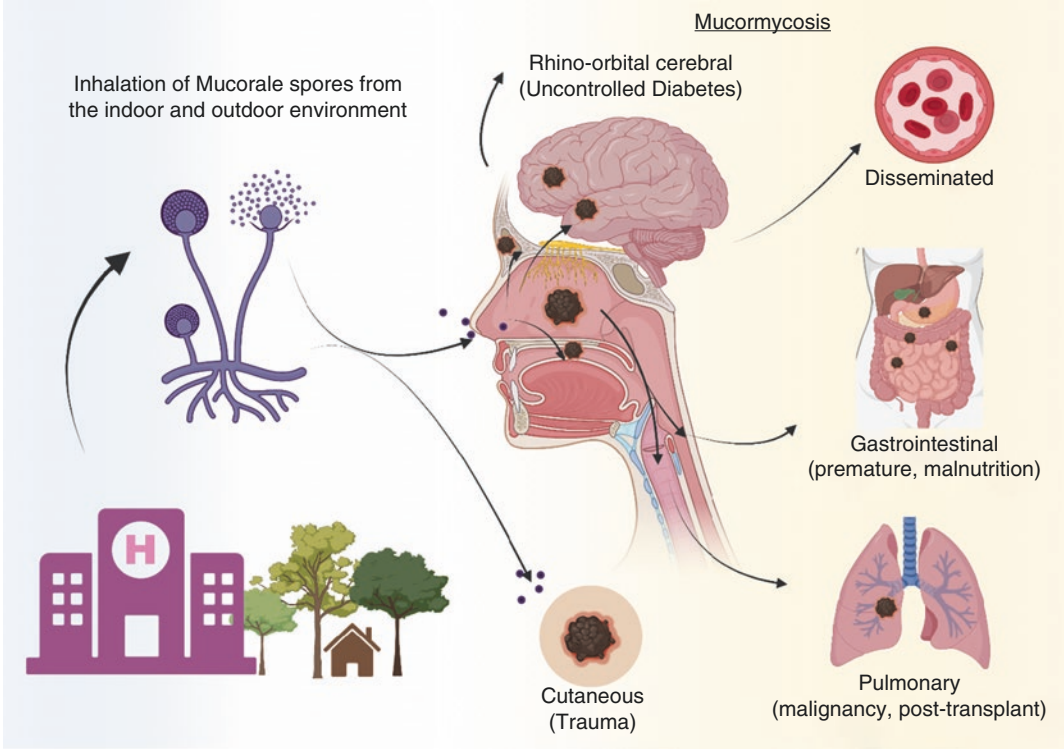

Fig. 4.1 Overview of pathogen entry and clinical presentations in mucormycosis

spp. can also attach to and invade the endothelium by recognizing a specific host receptor, the glucose-regulator protein 78 (GRP78) [33, 34]. This heat shock protein is a component of the host stress-related responses that helps in the specific binding of Mucorales germlings via the fungal ligands of the spore coat protein (CotH) family [35].

During SARS-CoV-2 infection, the endoplasmic reticulum stress is stimulated, which drives GRP78 synthesis [36]. In a recent study, nearly five times higher serum GRP78 was observed in patients with SARS-CoV-2 infection compared to the control group [37]. The GRP78 mediated attachment and internalization of the SARS-CoV-2 virus has also been reported [38]. Thus, upregulation of GRP78 enhanced by SARS-CoV-2 spike protein for its entry and by diffuse endothelial is seen in COVID-19 may also facilitate the attachment and invasion by Mucorales [39].

4.5 Immunopathogenesis

In a healthy host, the dormant/resting spores resist phagocytic killing but, swollen/germinating spores or hyphal forms are prone to degradation by immune cells. After endothelial penetration, Mucorales also encounter platelets that adhere to the spores and suppress germination, which contributes to hyphal damage, potentially hampering the fungal growth by adhering and damaging the hyphae or indirectly, by secreting inflammatory cytokines [40]. Various studies have investigated the interaction of Mucorales with host immunity (both innate and adaptive), and the details are summarized in Table 4.3.

The role of cytokines in modifying host response against the Mucorales originates from experimental studies, and its immunological interactions are depicted in Fig. 4.2.

Interleukin-1 beta (IL-1β) plays a pivotal role in response to pathogenic fungi with a significant role

Table 4.3 Interaction of the Mucorales with the immune system

Categories of the immune response with cellular type	Function	Immune evasion by Mucorales	References
Innate immune response			
Bronchial alveolar macrophages (BAM)	The first line of defense and prevents germination of Mucorale spores and has a role in iron restriction	• Inhibits phagosome maturation by cell wall melanin • Upregulation of iron acquisition genes • Upregulation of genes involved in nutrient assimilation to allow the use of alternate nutrients in a hostile macrophage environment	[32, 41–43]
Epithelial cells	The first line of defense and prevents the entrance of Mucorales spores	Mucorales damage the epithelial cells and upregulate epidermal growth factor receptor (EGFR) on the surface of epithelial cells, which promotes disease	[44, 45]
Polymorphonuclear leukocytes (PMNs) or neutrophil granulocytes	• Neutrophils produce cytokines to activate other immune cells • They can also form tight clusters around the spores containing them in structures resembling early granulomas • Neutrophil extracellular traps (NETs), reactive oxygen species and cationic peptides also damage both spore and hyphae	• Sialic acids in the cell wall protect against phagocytosis • Resting spores and the hyphae can reduce neutrophils chemotaxis	[46–48]
Natural killer (NK) cells	Natural killer (NK) cells recognize Mucorales and cause hyphal damage on activation	Mucorales hyphae have an immunosuppressive effect on dendritic cells and reduce the release of immunomodulatory molecules	[49–52]
Platelets	• Platelet recognition of Mucorales causes inhibition of spore germination and hyphal growth by secretion of various cytokines and chemokines • They also bind to and activate other immune cells	Excessive thrombosis seen in mucormycosis causes thrombocytopenia which also makes surgical interventions difficult	[40, 53]
Endothelial cells	Endothelial cells surround the innermost layer of blood vessels. It helps in hyphae recognition	The glucose-regulated protein 78 (GRP78) receptor present on endothelial cell surface facilitate angioinvasion by Mucorales	[33, 54, 55]
Dendritic cells (D.C.s)	• Dendritic cells link the innate immunity with the adaptive immunity • Production of IL23 by dendritic cells induces Th17 cells, which further promote neutrophil response	The resting (dormant) spores of Mucorales stimulate the maturation of dendritic cells	[56, 57]

(continued)

Table 4.3 (continued)

Categories of the immune response with cellular type	Function	Immune evasion by Mucorales	References
Adaptive immune response			
T cells	• Both CD4 and CD8 cells produce cytokines that mediate hyphal damage • Th17 cells produce IL 17, which promotes the antifungal defense of neutrophils • Release of interleukins (IL-4, IL-10, and IL-17) and IFNγ caused damage to hyphae		[58]

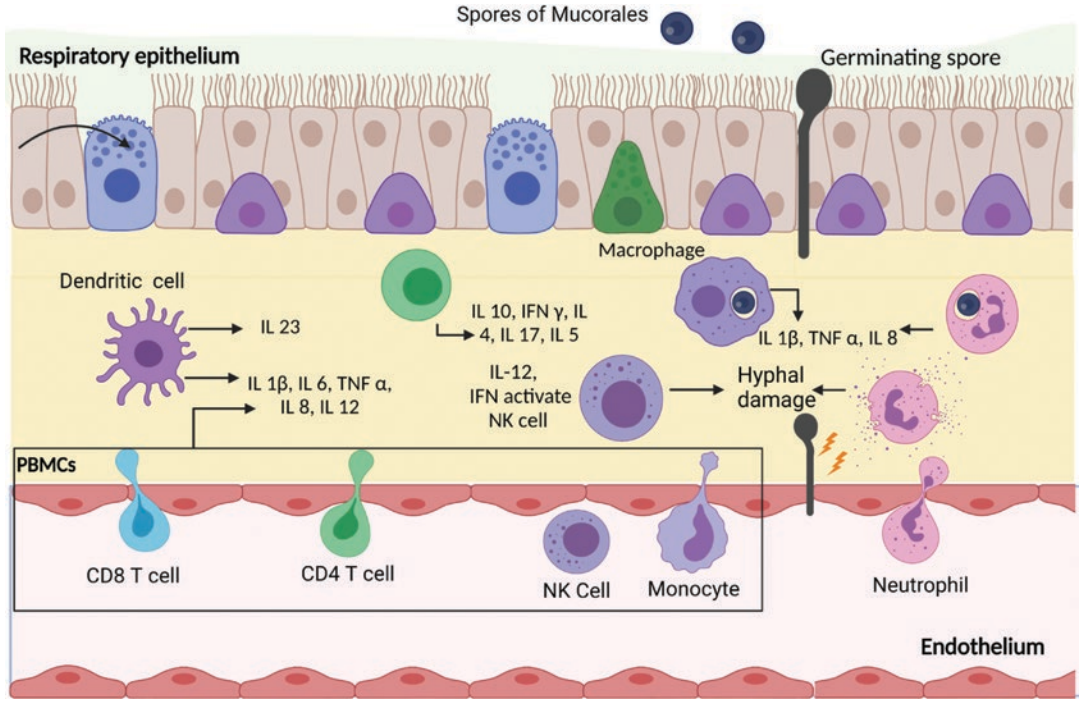

Fig. 4.2 Schematic representation of immune cells involved in the pathogenesis of mucormycosis

in the induction of other proinflammatory responses, hematopoiesis, Th17 cell differentiation, etc. [59]. Tumor necrosis factor α (TNF-α) has a dual role as an activator of inflammatory response and immunosuppression by mediating host apoptosis. At the same time, Interleukin-6 mediates leukocyte trafficking and production of acute-phase reactants while promoting T-cell proliferation and B cell responses [60]. The IL-12 promotes Th1 cell response, natural killer (N.K.) cell activation, Dendritic cell maturation, and production of interferon-γ (IFN-γ) and chemoattractant proteins like IL-8 also recruit immune cells, thereby mediating an effective immune response [60].

4.6 Factors Affecting the Pathogenesis of Mucormycosis in COVID-19

- *Hyperglycemia*: The SARS-CoV-2 impairs pancreatic beta-cell function and precipitates acute diabetic ketoacidosis (DKA) [61], and DKA has been was observed even with T2DM cases [62]. In the past, infections with SARS-CoV-1 have been seen to be associated with hyperglycemia persisting for as long as 3 years indicating long-term damage to pancreatic β-cells by these viruses [63]. Thus, SARS-CoV-2 mediated DKA could present even weeks or months after apparent recovery from the disease substantiating the late onset of CAM [14].
- *Corticosteroids*: Worsening of glycemic control can also occur secondary to the use of systemic steroids and antiviral agents in the management of COVID-19. A higher incidence of mucormycosis infections has been observed in patients who received corticosteroids during COVID-19 treatment, often in terms of inappropriate doses (≥ 6 mg/kg dexamethasone), duration (more than 10 days) or even when not indicated [14, 64]. Impaired macrophage and neutrophil function due to steroids explains suppressed antifungal immunity.
- *Iron metabolism*: Dysregulated iron metabolism is known to occur during COVID-19 [65]. The interaction between viral particles and hemoglobin perpetuates a cascade of dysfunctional hemoglobin synthesis, hemolysis, and heme accumulation with increased serum ferritin also seen in response to the inflammation [66–68]. The activation of macrophages and high IL-6 secretion also accentuates hyperferritinemia. This excessive intracellular free iron generates free radicals that cause endothelial destruction, leading to endothelitis, promoting fungal invasion [69].
- *Immune dysfunction*: The mucociliary clearance by the nasal epithelium is the primary innate immune defense against inhaled microorganisms. This clearance is delayed in COVID-19, allowing Mucorales spores to attach to the nasal epithelium [70]. Among innate immune cells, lymphopenia has been observed among COVID-19 patients, but its role in escalating the host susceptibility to mucormycosis is not clear due to insufficient data [71] (Fig. 4.3).

4.7 Common Misconceptions

The unprecedented epidemic of mucormycosis in the background of the COVID-19 pandemic has given rise to various misconceptions regarding CAM. The term “black fungus” has been used incorrectly and indiscriminately to indicate mucormycosis infection, which should be discouraged. Although the clinical presentation of mucormycosis comprises eschar and black necrotic lesions, the term “black fungus” actually denotes a completely different group of fungi that produce the pigment melanin, resulting in black hyphae, which are not seen in Mucorales. Another misconception is regarding the source of infection. The use of respirators, oxygenation during COVID-19 management, reuse of masks, etc. have been implicated as potential sources of infection, but this lacks scientific backing. There is also no evidence of human-to-human transmission, and this disease is acquired via Mucorales spores present ubiquitously in the environment, both indoors and outdoors. The prophylactic use of antifungals to prevent this infection in COVID-19 is also strongly discouraged since the incidence of infection is <10% in any COVID-19 cohort [8].

4.8 Future Perspectives

Although CAM is a new entity, various reports describing this infection have emerged over the past few months. Unfortunately, there is still a

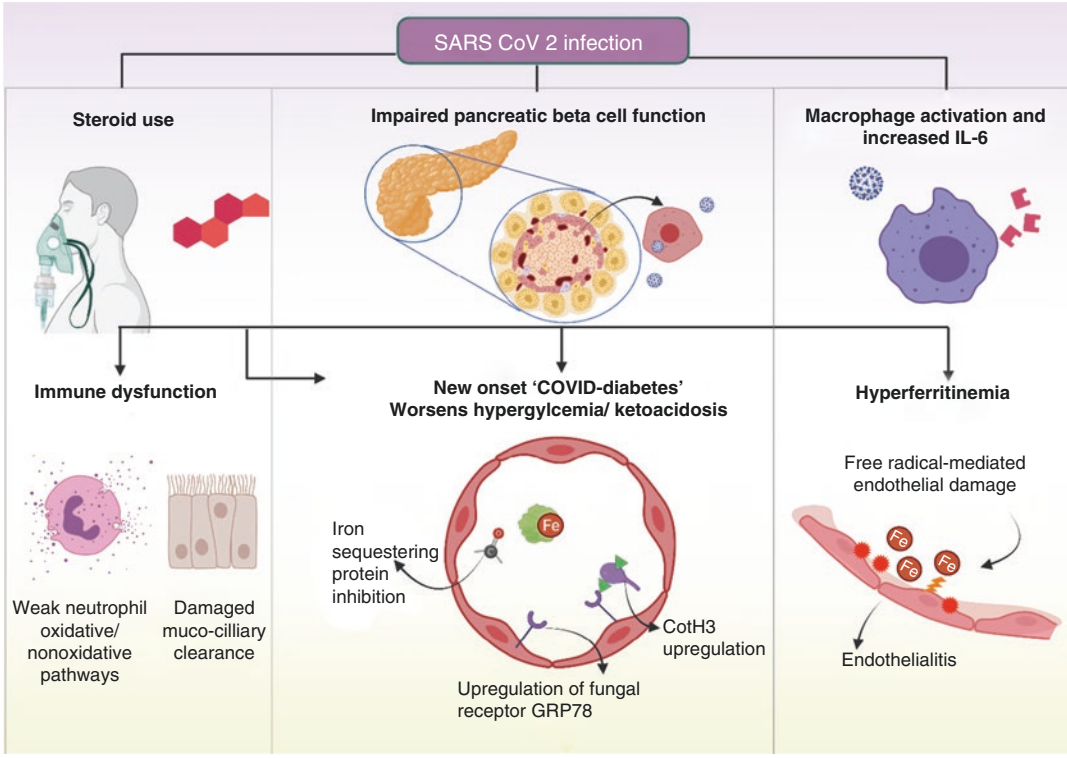

Fig. 4.3 Factors affecting pathogenesis of mucormycosis in patients with SARS CoV-2 infection

shortage of systematic prospective studies evaluating the various factors involved in disease pathogenesis and outcome, particularly in comparison to non-COVID mucormycosis cases. Studying the nature and extent of immunological dysfunction in COVID-19 and its impact on the pathogenesis of mucormycosis is a promising area of research.

4.9 Conclusion

Although host-immune response and inflammatory signalling against Mucorales have been established, the underlying molecular mechanism responsible for producing proinflammatory cytokines and activating inflammatory response against Mucorales is unknown. In the context of CAM, this field is even more unexplored. An understanding of the pathogenesis of CAM can provide new perspectives for developing novel diagnostic and treatment modalities.

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