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Candida albicans morphology dependent host FGF-2 response as a potential therapeutic target

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Abstract: Angiogenesis mediated by proteins such as Fibroblast Growth Factor – 2 (FGF-2) is a vital component of normal physiological processes and has also been implicated in contributing to disease state associated with various microbial infections. Previous studies by our group and others have shown that *Candida albicans*, a common agent of candidiasis, induces FGF-2 expression *in vitro*, and angiogenesis in brains and kidneys during systemic infections. However, the underlying mechanism(s) *via* which the fungus increases FGF-2 expression and the role(s) that FGF-2/angiogenesis plays in *C. albicans* disease remain unknown. Here we show, for the first time, that *C. albicans* hyphae (and not yeast cells) increase the FGF-2 response in human endothelial cells. Moreover, candidalysin, a toxin secreted exclusively by *C. albicans* in the hyphal state is required to induce this response. Our *in vivo* studies show that, in the systemic *C. albicans* infection model, mice treated with FGF-2 exhibit significantly higher mortality rates when compared to untreated mice not given the angiogenic growth factor. Even treatment with fluconazole could not fully rescue infected animals that were administered FGF-2. Our data suggest that the increase of FGF-2 production/angiogenesis induced by candidalysin contributes to the pathogenicity of *C. albicans*.

Keywords: angiogenesis; FGF-2; morphogenesis; candidalysin

1. Introduction

Candida albicans is a commensal/opportunistic fungal pathogen most commonly associated with mucosal diseases in humans. Lethal infections by *C. albicans* are continuously increasing in parallel with the growing proportion of vulnerable individuals such as immunocompromised patients and/or patients with indwelling medical devices [1-3]. In particular, disseminated infections pose a serious threat as mortality rates can exceed 40% even when patients receive antifungal therapy [4].

Many of the antifungal drugs that are currently used in clinical practice target fungal cells by inhibiting their growth or by killing them; they include azoles (targeting ergosterol synthesis), polyenes (physiochemically targeting ergosterol), and echinocandins (targeting cell wall synthesis) [5]. However, like many other microbial pathogens, *C. albicans* can develop resistance against antifungal drugs *via*: 1) mutations in drug target genes, 2) up-regulation of multi-drug resistance genes, or 3) the Hsp90-mediated stress response pathway [6,7]. Indeed, many clinical *C. albicans* isolates are multi-drug resistant and the emergence of drug resistance often leads to poor outcome in the treatment [8]. Therefore, it is imperative to develop novel therapeutic options that are not prone to resistance by the fungus.

Angiogenesis is the development of new blood vessels from pre-existing vessels [9]. It is regulated by proteins that either activate or inhibit the process [10]. Fibroblast Growth Factor-2 (FGF-2) is a pro-angiogenic protein that promotes angiogenesis in an autocrine fashion [11,12]. Studies have shown that FGF-2 is a more potent inducer of angiogenesis then other pro-angiogenic proteins such as Vascular Endothelial Growth Factor (VEGF) [13]. While modulation of FGF-2 or

 doi:10.20944/preprints201902.0025.v1

angiogenesis has become a common target in the treatment of cancer [14-16], studies from Ben-Ami *et al*., have shown such treatment could potentially be extended to treat fungal infections [17]. When *A. fumigatus* infected mice were treated with FGF-2 alone, or in combination with antifungals, there was a significant increase in survival rate, compared to the untreated group. However, the role of angiogenesis is understudied in *C. albicans* infection and disease progression.

Ashman *et al*., have shown that *C. albicans* increases angiogenesis in a murine model of systemic candidiasis [18]. Our group and others have revealed *in vitro* that *C. albicans* - mammalian host cell interactions result in increased production of proangiogenic growth factors, including FGF-2 and expression of the gene encoding VEGF [19-21]. However, the underlying molecular mechanisms of how the fungus induces FGF-2/angiogenesis in hosts remain elusive.

This study identifies a fungal factor that modulates host FGF-2 expression and investigates the role of FGF-2 in *C. albicans* infections. We found that *in vitro* induction of endothelial cell FGF-2 production is dependent on *C. albicans* morphology, with an increase in FGF-2 protein secretion only noted when the fungus was present in the filamentous form: Moreover, we determined that candidalysin, a secreted hypha specific toxin, regulates this process. Finally, using a murine model of systemic candidiasis, we discovered that treatment of *C. albicans* infected animals with FGF-2 results in increased mortality, suggesting that *C. albicans* induces FGF-2/angiogenesis to enhance pathogenicity.

2. Materials and Methods

2.1 Ethics statement

All animal experiments were performed in strict accordance with the guidelines of the UTSA Institutional Anicmal Care and Use Committee (IACUC) and in full compicance with the United States Animal Welfare Act (Public Law 98-198). National Institute of Health guidelines. The animal protocol used in this study were approved by the UTSA IACUC under protocol MU104. The experiments were conducted in the Division of Laboratory Animal Resources (DLAR) facilities that are acredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC).

2.2 Cell culture

Primary Human Umbilical Vein Endothelial Cells (HUVECs) were purchased from Lonza and seeded into T75 flask and maintained at 37°C + 5% CO_2 in Endothelial Cell Basal Medium containing hydrocortisone, abscorbic acid, Insulin growth factor, heparin, and FBS (EBM; Lonza) according to manufacturer instructions. Neither FGF-2, VEGF or gentamycin were added to the medium. Confluent flasks were trypsinized and seeded into a 96 well plate for challenge with *C. albicans*.

2.3 Fungal strains and growth conditions

C. albicans strains were maintained as glycerol stocks at -80°C and were propagated by streaking on yeast-peptone-dextrose (YPD) agar plates as needed. A day before the infection (as explained below) colonies of cells from the plate were transferred to YPD liquid medium and were incubated overnight (16 hours) at 30°C with shaking (180 rpm). The yeast cells were then washed with sterile phosphate-buffered saline (PBS), resuspended, and diluted to the desired concentration using sterile PBS. The list of strains used in this study are shown in Table 1.

2.4 HUVECs challenge with C. albicans

HUVECs were counted and the cell number was adjusted to seed 5 x 10^3 cells/well in 100 µl medium in a 96-well plate. HUVECs were challenged with 5 x 10^4 cells/well (10 µl/well in PBS) of

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each of the *C. albicans* strains for 24 hours. The supernatants were collected from each well and FGF-2 Enzyme Linked Immuno Sorbent Assay (ELISA; R&D systems) was performed according to manufacturer instructions to determine FGF-2 protein levels. When tetracycline regulatable strains were used, doxycline were added to appropriate wells at a final concentration of 20 µg/ml [22]. For experiments involving compound 9029936 [23], the compound was added to appropriate wells in various concentrations as explained in results section *3.1*. All ELISA measurements were performed in at least two independent experiments with three technical repeats.

Table 1 List of strains

Strain	Reference
SC5314	[24]
CAN 33 (*efg1 Δ/Δ*)	[25]
SSY50-B (*tet-NRG1*)	[26]
SN152	[27]
17322 (*als3 Δ/Δ*)	[28]
bcr1 Δ/Δ	[29]
BWP17 + CIp 31	[30]
ece1 Δ/Δ	[30]
ece1 Δ + ECE1	[30]
ece1 Δ + ECE1$_{184-257}$	[30]
SAP456MS4A/B (*Sap 4-6Δ*)	[31]
SAP123MS4C/D (*Sap 1-3Δ*)	[31]

2.4 HUVECs challenge with C. albicans spent medium

The concentration of *C. albicans* strain SC5314 (with or without compound 9029936) and the yeast-locked mutant *efg1Δ/Δ [29]* were adjusted to 5 x 10^6/ml and were grown for 24 hours in HUVEC cell culture medium (EBM) at 37°C. The medium was centrifuged at 2500 x g for 15 minutes and the supernatant filtered through a sterile 0.22 µm filter. The resulting medium was immediately added to HUVECs and incubated for 24 hours before proceeding to the ELISA.

2.5 HUVECs challenge with C. albicans non-viable (heat-killed and PFA-treated) strains

Wild-type SC5314 and *efg1 Δ/Δ* mutant strains were resuspended at a final concentration of 5 x 10^6/ml in EBM and incubated at 37°C until germination was observed (~3 hrs) in the tubes containing

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the SC5314 strain. As a control, we also incubated both *C. albicans* strains at 28°C (no germ tube control). The vials containing the cells were then subjected to heat (95°C) for 30 minutes [32]. Since heat treatment could alter the cell wall of *C. albicans*, we also included a paraformaldehyde (PFA)-treated group. For the PFA treatment, the fungal cells were incubated in PFA for 30 minutes then washed with PBS several times. For the challenge experiment, either 5 x 10^4 viable or non-viable (heat-killed or PFA-treated) cells were added to each HUVEC well and incubated for 24 hours. Both viable and non-viable *C. albicans* were also plated on YPD plates to confirm the loss of viability in the heat and PFA treated groups.

2.6 HUVECs challenge with candidalysin peptide

Candidalysin peptide (SIIGIIMGILGNIPQVIQIIMSIVKAFKGNK) [30] was a kind gift from Dr. Julian Naglik (King's College London). The peptide were prepared as a 10 mg/ml stock in sterile water. Serum-starved HUVECs were seeded in a 96 well plate and incubated with candidalysin peptide diluted to 9 μM in sterile water for 24 hours. Supernatants were collected to perform ELISA.

2.7 Evaluation of FGF-2 monotherapy and combination therapy with fluconazole in a murine model of systemic candidiasis

Balb/C mice were infected with 1 x 10^6 SC5314 yeast cells in 100 μl sterile PBS *via* a tail-vein. For monotherapy, the infected mice were given 1.6 μg of recombinant human FGF-2 (R&D systems) in 100 μl sterile PBS intravenously at 3 and 5 hours after infection [17]. For combination therapy, fluconazole was administered daily at 0.5 mg/kg *via* the intraperitoneal route starting 5 hours after infection for 7 days. We also included animal groups infected with SC5314 only or SC5314 treated with fluconazole. Negative controls included non-*Candida* groups given either PBS or rFGF-2 only. Post-infection survival of the mice was monitored twice daily, and body weights were measured once daily (as they indicate mice health). Differences between the survival curves were evaluated for significance using the Kaplan-Meier test.

2.7 Statistics

Prism (GraphPad Software Inc.) was used to perform statistical analysis. $P \leq 0.05$ was considered to be statistically significant. All *in vitro* experiments were performed in tripicate, and each experiment was performed on at least three separate occasions. Data was expressed as mean ± SEM. *In vivo* challenge experiments were performed on two independent occasions with n = 5 in each group.

3. Results

3.1. FGF-2 protein secretion from hosts is dependent on the morphology of the C. albicans

In the current study, HUVECs were challenged with either a wild-type (SC5314), a yeast - locked mutant (*efg1 Δ/Δ*), or with a regulatable *tet-NRG1* strain [26]. Nrg1 is a transcriptional repressor which suppresses the expression of hypha specific genes in yeast form cells. The *tet-NRG1* strain was constructed by placing one allele of *NRG1* under the control of a tetracycline-regulatable promoter so that morphology can be manipulated by adding or omitting doxycycline (dox)[26]. While addition of dox permits filamentation, omission results in a yeast - locked morphology. As shown in Figure 1a, compared to the uninfected PBS control (mock), FGF-2 production was significantly higher only when *C. albicans* was able to filament – WT & *tet-NRG1* (+dox). We did not observe a substantial change in HUVECs challenged with the *efg1 Δ/Δ* and *tet-NRG1* (-dox) strains (when *C. albicans* cells remained in the yeast form). As a control, an ELISA was also performed on supernatants from wells with *C. albicans* only (no HUVEC), however as expected no FGF-2 expression was noted (data not shown).

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As an alternative approach to test how *C. albicans* morphology affects FGF-2 production, we challenged HUVEC cultures with the *C. albicans* WT strain SC5314 in the absence or presence of compound 9029936 for 24 hours. Compound 9029936, identifed by Romo *et al*., blocks *C. albicans* filamentation and biofilm formation [23]. In agreement with the data represented in Figure 1a, HUVECs responded to untreated *C. albicans* (hyphae) with increased FGF-2 expression, however the presence of the drug (2.5 to 40 μM) significantly diminished this effect (Figure 1b). Compound 9029936 by itself does not affect FGF-2 response from HUVECs (Figure S1). Taken together (Figure 1a and 1b), these results suggest that the host FGF-2 response is specific to the hyphal form of *C. albicans*.

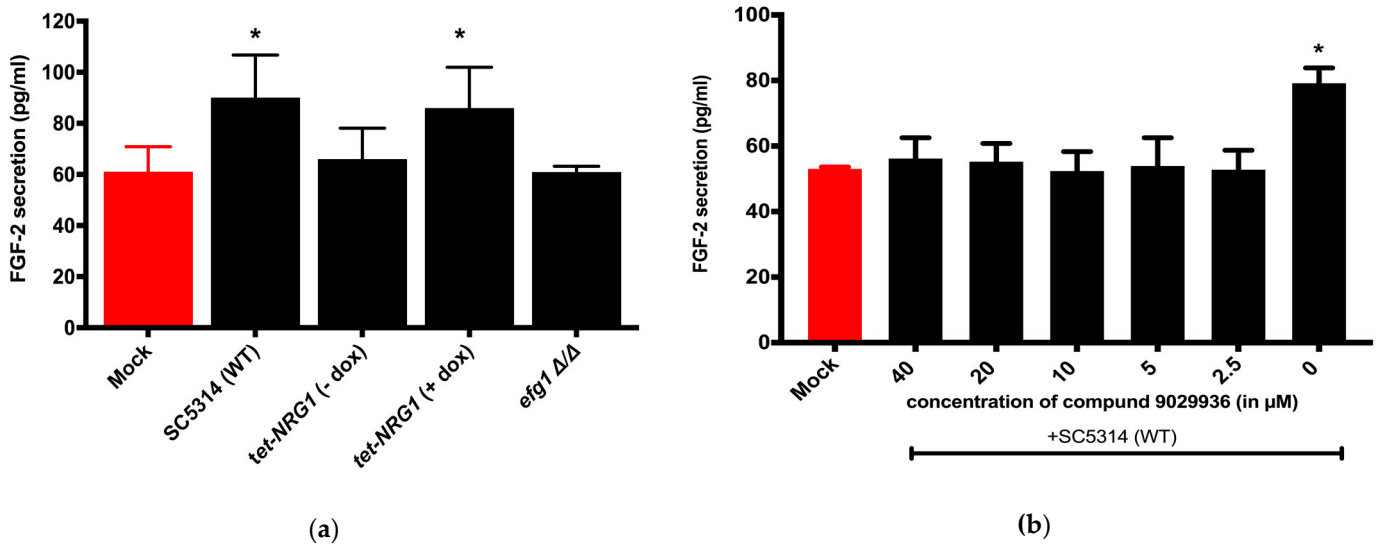

Figure 1. Host FGF-2 response is dependent on the morphology of *C. albicans*. a) HUVECs were challenged with SC5314 (WT), *tet-NRG1* (+/- dox), or *efg1*Δ/Δ strains for 24 hours. FGF-2 levels were measured using ELISA. One-way ANOVA was significant (P = 0.0005). Dunnett's multiple comparison was used to compare each infected group with the uninfected group (mock). A P < 0.05 was considered to be significant, as indicated by (*). b) HUVECs were challenged with the wild-type strain SC5314 in the presence (40 to 2.5 μM) or absence (0 μM) of compound 9029936 for 24 hours and the amount of FGF-2 measured in the supernatants. One-way ANOVA was significant (P = 0.042). Dunnett's multiple comparison test demonstrated a statistically significant difference (*P<0.05) only between the Mock and infected group without compound 9029936 treatment (0 μM).

3.2. Viable C. albicans hyphae are required for the induction of the FGF-2 response

We next determined whether the FGF-2 production/induction is dependent on the viability of the fungal cells. To that end, *C. albicans* WT (SC5314) were allowed to germinate by incubating at 37°C in EBM, and then the hyphae were heat-inactivated/PFA-treated (non-viable) and added to HUVEC wells for 24 hours. As shown in Figure 2, while live (no heat or PFA treatment) *C. albicans* hyphae were able to induce statistically significant FGF-2 protein production, HUVECs challenged with non-viable *C. albicans* hyphae do not display this phenomenon when compared to untreated controls (mock). We also did not observe any difference in FGF-2 secretion between HUVECs challenged with non-viable WT and viable/non-viable *efg1*Δ/Δ. The control group which were incubated at 28°C did not any show any germ tube formation. When the live counterparts of this group were added to HUVECs and incubated at 37°C for 24 hours, as expected they switched to hyphal growth and induced significant increase in FGF-2 expression when compared to its heat-killed/PFA-treated counterparts which cannot make hyphae due to lack of viability. These results suggest that a factor produced from live *C. albicans* hyphae is required to elicit an FGF-2 response.

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Once it was confirmed that *C. albicans* hyphae were required to elicit an FGF-2 host response, we wanted to test if hyphal wall associated proteins are involved in this process. Als3, a member of agglutinin-like sequence (Als) family of proteins, expressed on the hyphal surface is required for *C. albicans* adhesion and invasion of epithelial and endothelial cells [33]. Bcr1 is a transcriptional regulator of several hyphal cell wall proteins, including the Als3 invasin [34]. We tested *als3* and *bcr1* mutant strains for their ability to induce FGF-2 production but we observed no significant change when compared to their respective parental strains (Figure S2). We also tested the possible role of secreted aspartyl proteases (Saps; suggested to play a role in *C. albicans* virulence) in modulating the host FGF-2 response. To that end, HUVECs were challenged with mutant strains lacking either the Saps 1-3 or 4-6 subfamilies or with the WT strain; however, deletion of the Sap subfamilies does not impact FGF-2 production from HUVECs in a 24 hour period (Figure S3).

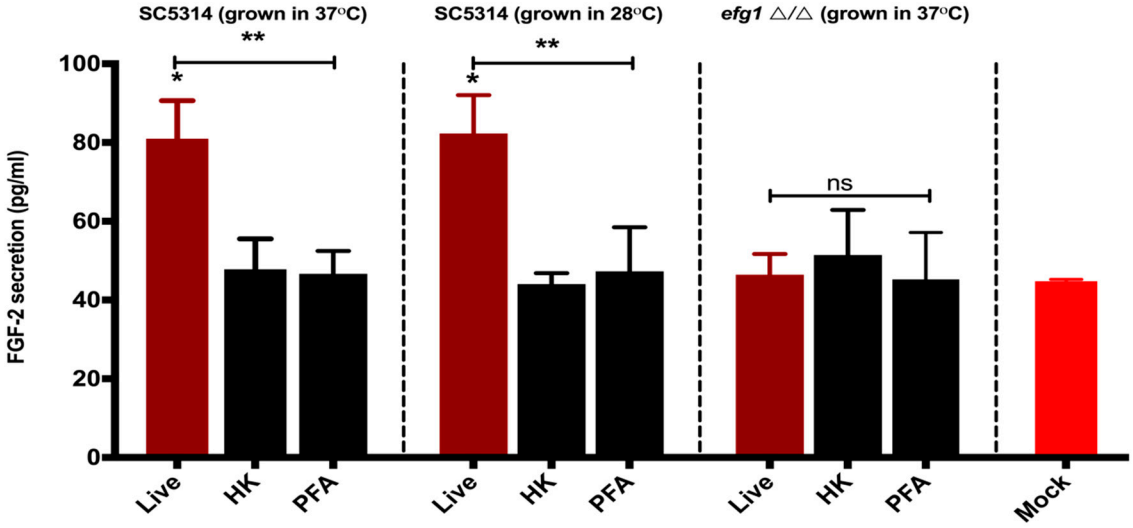

Figure 2. Factor(s) from viable *C. albicans* hyphae regulates the host FGF-2 response. *C. albicans* SC5314 (WT) strain was grown in EBM at 37°C until germ tubes were visible (~3 hours). Controls were SC5314 grown at 28°C and the *efg1*Δ/Δ mutant at 37°C for the same time period (during which no germ tubes were detected). Each group was split into three sub-groups – no treatment (live), or treatment with heat (HK) or paraformaldehyde (PFA). HUVECs were challenged with live, HK or PFA-treated *C. albicans* and were maintained at 37°C for 24 hours. FGF-2 levels in the culture supernatants were measured by ELISA. Both one-way ANOVA (P = 0.009) and Dunnett's multiple comparison test (P<0.05) showed statistically significant differences between the Mock and SC5314 live groups (as indicated by *). There was also a statistically significant difference between the live and non-viable groups (HK and PFA) in SC5314 groups (as indicated by **).

3.3. Candidalysin induces host FGF-2 protein secretion

Candidalysin, encoded by the *ECE1* gene, is a 31 amino acid peptide and the first cytolytic toxin identified in *C. albicans* [30]. Since, candidalysin is a hypha specific toxin, and was found to be a key factor involved in activating specific host responses, we decided to test if candidalysin also regulates the host FGF-2 response. We used several *ECE1* gene modified strains to evaluate the relationship between candidalysin and the host FGF-2 response. HUVECs were challenged with a *C. albicans ECE1* null mutant in which both copies of the gene were deleted (*ece1*Δ/Δ) or with a strain in which one copy of full-length allele of *ECE1* was restored (*ece1*Δ/Δ + *ECE1*), or the strain lacking the region of *ECE1* that encodes candidalysin (*ece1*Δ/Δ + *ECE1*$_{\Delta 184\text{-}257}$), or the wild-type parental strain (BWP17 + CIp30) for 24 hours. As shown in Figure 3a, while the null *ece1 Δ/Δ* mutant and the *ece1*Δ/Δ + *ECE1*$_{\Delta 184\text{-}257}$ strain failed to cause a substantial increase in FGF-2 secretion, the parental and reconstituted *ece1*Δ/Δ + *ECE1* strains induced a significant increase when compared to the uninfected control (mock).

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To verify that candidalysin is the mediator of FGF-2 response, we challenged HUVEC with the candidalysin peptide and, as shown in Figure 3b, addition of the peptide alone was sufficient to induce a significant increase in FGF-2 secretion when compared to the untreated vehicle control (sterile water). Thus, findings from Figure 3a and 3b confirm that candidalysin induces secretion of FGF-2 from endothelial cells.

To determine if the spent medium from *C. albicans* is sufficient to induce FGF-2 secretion, the *C. albicans efg1*Δ/Δ mutant and the SC5314 wild-type strain (with or without 10 μM compound 9029936) were grown in EBM for 24 hours. The medium was filtered, then added to HUVECs and incubated for 24 hours. However, we did not observe a significant difference in FGF-2 production among the groups (Figure S4).

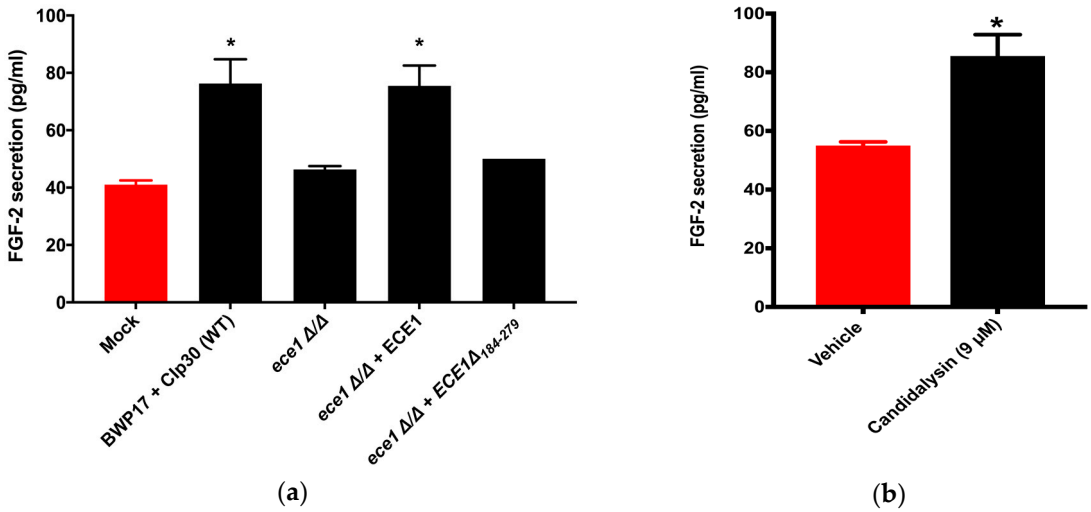

Figure 3. Candidalysin regulates host endothelial FGF-2 response. a) HUVECs were challenged with BWP17 + CIp30 (WT), *ece1*Δ/Δ, *ece1*Δ/Δ + *ECE1*, or *ece1*Δ/Δ + *ECE1*$_{\Delta 184\text{-}257}$ for 24 hours. FGF-2 levels were measured using ELISA. One-way ANOVA was significant (P = 0.001). Dunnett's multiple comparison demonstrated that, compared to the uninfected group (Mock), only HUVECs challenged with BWP17 + CIp30 or *ece1*Δ/Δ + ECE1 show a statistically significant increase in the FGF-2 response (P < 0.05), as indicated by (*). b) HUVECs were treated with vehicle control (water) or candidalysin peptide for 24 hours. Two-tailed T-test showed a statistically significant difference between the two groups (P = 0.049), indicated as (*).

3.4. FGF-2 enhances mortality rate in a murine model of systemic candidiasis

To evaluate the role of FGF-2/angiogenesis in systemic candidiasis, we infected mice with the wild-type strain SC5314 *via* a tail vein either alone or in addition to two FGF-2 (1.6 μg) treatments (at 3 hours and 5 hours post-infection). The other treatment groups were – infection + fluconazole (0.5 mg/kg) treatment for seven days, or infection + a combination of fluconazole and FGF-2. The infected group treated with FGF-2 showed a significant increase in the mortality rate which is evident by all mice reaching death by day 6, compared to the no treatment group, in which the animals start succumbing from day 6. (Figure 4). Interestingly, co-treating the infected mice with both FGF-2 and fluconazole resulted in increased mortality rates when compared to the infected mice group treated with fluconazole alone. Once the fluconazole treatment was stopped, we observed a decrease in survival rate from 100% to 80% on day 12 in WT + fluconazole group (not shown in figure). Treatment of uninfected mice with PBS or FGF-2 did not have any impact on the mortality (survival rates) or morbidity (body weights) of the animals (data not shown).

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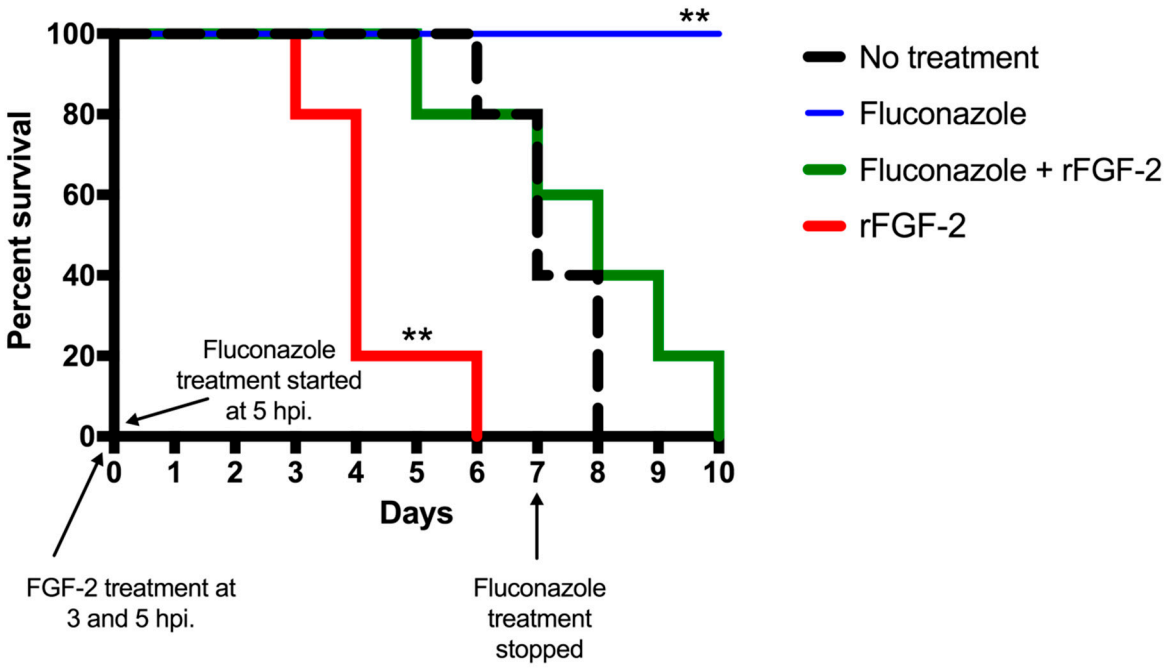

Figure 4. Treatment with FGF-2 increases the mortality rate in a murine model of systemic candidiasis. Balb/C mice were challenged with 1x10^6 SC5314 yeast cells *via* a tail vein. Fluconazole was administered daily 0.5 mg/kg *via* the IP route from the day of infection for 7 days in the respective animal groups. Recombinant FGF-2 (1.6 µg each dose?/mouse) was administered intravenously in the respective groups 3 hours and 5 hours post-infection. Log-rank (Mantel-Cox) test was statistically significant (P<0.0001) between which groups?. A pair-wise comparison was also performed to compare no treatment vs rFGF-2 (**P=0.006), no treatment vs rFGF-2 + Fluconazole (P=0.291), or no treatment vs Fluconazole (**P=0.002), as indicated on the graph. Once the fluconazole treatment was stopped the survival rate of the Fluconazole group changed to 80% (with the death of 1 of the mice) on day 12 post-infection (not shown on graph).

4. Discussion

Understanding host-pathogen interactions provides a fundamental platform for the development of new therapeutic interventions. Our previous studies revealed an intriguing outcome of the host-pathogen interaction: the induction of the pro-angiogenic growth factor FGF-2 in mammalian hosts by fungal pathogens, including *C. albicans* [19,20]. Our findings are congruent with the study by Ashman *et al*, where they observed an increase in angiogenesis, characterized by enhanced endothelial cell proliferation including formations of capillary buds and small blood vessels in brain and the kidney sections, in a murine model of systemic candidiasis [18]. However, the underlying mechanisms of the *C. albicans* - host FGF-2/angiogenesis response has not yet been fully explored.

C. albicans can grow in several different morphologies and it is the switch from the yeast to hyphal form which is most closely linked to its capacity to cause disease. During pathogenesis, while the yeast morphology is essential for colonization and dissemination through the bloodstream, the hyphal form is required for invasion and damage to host tissues [35,36]. During host - *C. albicans* interactions, the host response has been shown to vary depending on the morphological form of the fungus [37-39]. This prompted us to test whether the induction of host FGF-2 secretion is also linked with a particular morphological form of *C. albicans*. To this end, we used HUVECs because they recapitulate the features of endothelial cells lining the lumen of blood vessels [40]. Moreover, HUVECs have already been extensively used to characterize endothelial cell FGF-2 expression [41], and to investigate *C. albicans*-endothelial cell interactions [42-45]. As the results in Figure 1a and 1b show, *C. albicans* can induce FGF-2 secretion only in the hyphal form.

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Previous studies have shown that the host response may vary depending on the viability of the *C. albicans* [46-48]. We therefore compared the host FGF-2 response from endothelial cells challenged with both viable and non-viable hyphae. The increase in FGF-2 response was specific to live/viable hyphae (Figure 2). Fungal invasion of host cells is a hallmark of *C. albicans* infection, and Als3 is a key mediator of *C. albicans* invasion of epithelium and endothelium [33]. Our findings suggest that the invasins (and definitely Als3) are not responsible for inducing the host FGF-2 response (Figure S2). Secreted aspartyl proteases are virulent traits of *C. albicans* which are essential for degradation of host proteins and promote *C. albicans* invasion/penetration of host cells [49]. However, we found that the Saps sub-families 1-3 or 4-6 are also not involved in inducing the host FGF-2 response (Figure S3). Based on these findings (Figure 2, S2, and S3), we hypothesized that a hypha-specific factor produced by *C. albicans* – after or during invasion - is involved in eliciting the FGF-2 response.

Candidalysin is a *C. albicans* hypha-specific toxin encoded by *ECE1* [30]. Although the *ece1* mutant strains can still form hyphae and invade host epithelium, studies have shown that these mutants fail to cause epithelial cell damage and activate pro-inflammatory signaling (danger) pathways in oropharyngeal and vulvovaginal candidiasis [30,50,51]. This indicates that this toxin can regulate specific host responses. Studies from *A. fumigatus* show that it produces a toxin (gliotoxin) that regulates host angiogenesis [52]. Therefore, we hypothesized that candidalysin might regulate the host FGF-2 response. Indeed, as the results in Figure 3 show, *C. albicans* induces FGF-2 secretion only when it can produce functional candidalysin., However, when spent medium from *C. albicans* hyphal cultures was added to HUVECs, we did not observe a considerable change in FGF-2 secretion (Figure S4): this may be due to candidalysin instability in the culture medium. This would be consistent with the recent observation that epithelial cells challenged with *C. albicans* hyphae, they were damaged, but when the exhausted culture medium was used to infect a fresh epithelial culture, no damage was observed (discussed in [53]). Nevertheless, our results strongly support the assertion that candidalysin is primarily responsible for eliciting the host FGF-2 response. While prior studies have been dedicated to elucidating the relationship between candidalysin and host epithelium responses, not much was known about the regulation of endothelial cell responses by candidalysin. Here we provide the earliest evidence that candidalysin also plays a role in regulating/mediating endothelial cell responses. More studies will be required to determine whether candidalysin also plays a role in any other endothelial cell responses.

What is the consequence of the induction of proangiogenic growth factors during C. albicans infections? Previous studies have shown that in microbial infections host angiogenesis can either enhance host-defense mechanisms or contribute to pathogenicity. For example, treatment with FGF-2 improves antifungal drug activity in a murine model of aspergillosis [17]. Also, ribonuclease 5 angiogenin is known to possess microbicidal activity against pathogenic bacteria in the gastrointestinal tract [54]. In contrast, recent findings have demonstrated that tuberculosis bacteria induce granuloma-associated angiogenesis, which contributes to its pathogenesis [55,56]. Another study found the formation of capillary buds and blood vessels around *C. albicans* foci within infected brains and kidneys [18]. Our discovery that the administration of recombinant FGF-2 into mice post infection with *C. albicans*, significantly increased mortality compared to non-FGF-2 treated animals, suggests that angiogenesis mediated by FGF-2 enhances the pathogenicity of *C. albicans* during a disseminated infection.

From the current study, it appears that angiogenesis is harmful to the host in disseminated *C. albicans* infections; however, the precise mechanism by which angiogenesis enhances the pathogenicity of the fungus remains unclear. Both blocking of FGF-2 with a neutralization antibody or addition of recombinant FGF-2 does not impact the ability of *C. albicans* to damage host endothelial cells *in vitro* (Vellanki and Lee, Unpublished data). We also found that addition or neutralization of cytokines such as IL-8 by a drug or antibody did not affect endothelial cell FGF-2 response in *C. albicans* infections (Vellanki and Lee, Unpublished data) Based on these results, we hypothesize that FGF-2/angiogenesis is a direct response induced by *C. albicans* to enhance its

dissemination into the deeper host tissues, as previously observed in *Mycobacterium tuberculosis* infections [57]. Our future studies will focus on elucidating the extent to which *C. albicans* hyphae and candidalysin contribute to increasing host angiogenesis in the murine systemic candidiasis model by using *ECE1* mutants that make hyphae but do not secrete candidalysin and, if required, by also expressing candidalysin in a yeast-locked mutant.

The emergence of antifungal drug resistance has limited our ability to treat *C. albicans* infections and often leads to poor clinical outcomes. Thus, the identification of novel therapeutic avenues that can be exploited to treat *C. albicans* infection is required. Host-directed therapeutic approaches which enhance the host's ability to fight the infection, rather than targeting the *C. albicans* components have already been investigated [58]. As FGF-2 secretion/angiogenesis is apparently enhanced during a *C. albicans* infection, this process could be targeted by inhibiting FGF-2 function. Blocking a host response related to fungal pathogenicity will represent a new paradigm for treating fungal infections as they can be used irrespective of *Candida* resistance to current antifungals. Moreover, by targeting a host component of the disease process, the risk of the fungus developing a resistant mechanism to overcome this, is massively reduced. Systemic *Candida* infections pose a serious risk, with high mortality rates; thus, a new approach to block host FGF-2 functions during systemic infections could be an effective treatment option. There are drugs which inhibit angiogenesis and/or block FGF-2 function [14], and some of them are already approved by the FDA to treat other diseases. Successful demonstration of efficacy of any of these drugs would eliminate the time normally needed for drug development and enable them to be far more rapidly applied to patients with systemic/disseminated *candidiasis*. Intriguingly, during our preliminary studies, we discovered that *C. auris* - in which candidalysin has not been identified - also enhanced the secretion of FGF-2 from host cells (Vellanki and Lee, unpublished data). This approach, therefore, could potentially be extended to disseminated infections caused by other *Candida* species.

Supplementary Materials: The following are available online, Figure S1: Compound 9029936 itself does not affect endothelial cell FGF-2 response. Figure S2: *Als3 and BCR1*-regulated cell wall proteins are not involved in the regulation of host FGF-2 secretion. Figure S3: Sap proteins are not linked with the host endothelial FGF-2 response. Figure S4: *C. albicans* spent medium is not sufficient to induce the host FGF-2 response.

Author Contributions: "conceptualization, S.C.L. and S.V..; methodology, S.C.L. and S.P.S.; software, none.; validation, S.V., E.Y.H. and S.C.L.; formal analysis, S.V. and S.C.L.; investigation, S.V. and E.Y.H.; resources, S.C.L. and S.P.S. ; data curation, S.C.L..; writing—original draft preparation, S.V. and S.C.L..; writing—review and editing, S.C.L., S.V., S.P.S and E.Y.H..; visualization, S.V. and S.C.L.; supervision, S.C.L.; project administration, S.C.L., S.V. and E.Y.H.; funding acquisition, S.C.L.".

Funding: This work was supported by NIH/NIAID R03 AI11917 and UTSA Research funds to S.C.L.

Acknowledgments: We would like to acknowledge Dr. Brian Peters (The University of Tennessee Health Science Center), Dr. Duncan Wilson (University of Aberdeen) and Dr. Julian Naglik (King's College London) for providing us with Sap and candidalysin mutant strains and the candidalysin peptide. We are indebted to Drs. Jose Lopez-Ribot, Chiung-Yu Hung, Astrid Cardona, and Ashraf Ibrahim (Harbor-UCLA Medical Center) for valuable discussions. We would also like to thank the UTSA medical mycology and STCIED groups for their critiques.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

5. References

1. Brown, G.D.; Denning, D.W.; Gow, N.A.; Levitz, S.M.; Netea, M.G.; White, T.C. Hidden killers: human fungal infections. *Sci Transl Med* **2012**, *4*, 165rv113. [CrossRef] [PubMed]
2. Carpino, N.; Naseem, S.; Frank, D.M.; Konopka, J.B. Modulating host signaling pathways to promote resistance to infection by *Candida albicans*. *Front Cell Infect Microbiol* **2017**, *7*, 481. [CrossRef] [PubMed]

3. Pfaller, M.A.; Diekema, D.J. Epidemiology of invasive candidiasis: a persistent public health problem. *Clin Microbiol Rev* **2007**, *20*, 133-163. [CrossRef] [PubMed]
4. Kullberg, B.J.; Arendrup, M.C. Invasive Candidiasis. *N Engl J Med* **2015**, *373*, 1445-1456. [CrossRef]
5. Nett, J.E.; Andes, D.R. Antifungal Agents: Spectrum of activity, pharmacology, and clinical indications. *Infect Dis Clin North Am* **2016**, *30*, 51-83. [CrossRef] [PubMed]
6. Shapiro, R.S.; Robbins, N.; Cowen, L.E. Regulatory circuitry governing fungal development, drug resistance, and disease. *Microbiol Mol Biol Rev* **2011**, *75*, 213-267. [CrossRef] [PubMed]
7. Shapiro, R.S.; Uppuluri, P.; Zaas, A.K.; Collins, C.; Senn, H.; Perfect, J.R.; Heitman, J.; Cowen, L.E. Hsp90 orchestrates temperature-dependent *Candida albicans* morphogenesis via Ras1-PKA signaling. *Curr Biol* **2009**, *19*, 621-629. [CrossRef] [PubMed]
8. Sanguinetti, M.; Posteraro, B.; Lass-Florl, C. Antifungal drug resistance among *Candida* species: mechanisms and clinical impact. *Mycoses* **2015**, *58 Suppl 2*, 2-13. [CrossRef] [PubMed]
9. Folkman, J.; Klagsbrun, M. Angiogenic factors. *Science* **1987**, *235*, 442. [PubMed]
10. Nishida, N.; Yano, H.; Nishida, T.; Kamura, T.; Kojiro, M. Angiogenesis in cancer. *Vasc Health Risk Manag* **2006**, *2*, 213-219. [PubMed]
11. Sato, Y.; Rifkin, D.B. Autocrine activities of basic fibroblast growth factor: regulation of endothelial cell movement, plasminogen activator synthesis, and DNA synthesis. *J Cell Biol* **1988**, *107*, 1199-1205. [PubMed]
12. Seghezzi, G.; Patel, S.; Ren, C.J.; Gualandris, A.; Pintucci, G.; Robbins, E.S.; Shapiro, R.L.; Galloway, A.C.; Rifkin, D.B.; Mignatti, P. Fibroblast Growth Factor-2 (FGF-2) induces Vascular Endothelial Growth Factor (VEGF) expression in the endothelial cells of forming capillaries: An autocrine mechanism contributing to angiogenesis. *J Cell Biol* **1998**, *141*, 1659. [PubMed]
13. Pepper, M.S.; Ferrara, N.; Orci, L.; Montesano, R. Potent synergism between vascular endothelial growth factor and basic fibroblast growth factor in the induction of angiogenesis *in vitro*. *Biochem Biophys Res Commun* **1992**, *189*, 824-831. [PubMed]
14. Akl, M.R.; Nagpal, P.; Ayoub, N.M.; Tai, B.; Prabhu, S.A.; Capac, C.M.; Gliksman, M.; Goy, A.; Suh, K.S. Molecular and clinical significance of fibroblast growth factor 2 (FGF2 /bFGF) in malignancies of solid and hematological cancers for personalized therapies. *Oncotarget* **2016**, *7*, 44735-44762. [CrossRef] [PubMed]
15. Metzner, T.; Bedeir, A.; Held, G.; Peter-Vörösmarty, B.; Ghassemi, S.; Heinzle, C.; Spiegl-Kreinecker, S.; Marian, B.; Holzmann, K.; Grasl-Kraupp, B., et al. Fibroblast Growth Factor receptors as therapeutic targets in human melanoma: synergism with BRAF inhibition. *J Invest Dermatol* **2011**, *131*, 2087-2095. [CrossRef] [PubMed]
16. Brady, N.; Chuntova, P.; Bade, L.K.; Schwertfeger, K.L. The FGF/FGFR axis as a therapeutic target in breast cancer. *Expert Rev Endocrinol Metab* **2013**, *8*, 391-402. [CrossRef] [PubMed]
17. Ben-Ami, R.; Albert, N.D.; Lewis, R.E.; Kontoyiannis, D.P. Proangiogenic growth factors potentiate in situ angiogenesis and enhance antifungal drug activity in murine invasive aspergillosis. *J Infect Dis* **2013**, *207*, 1066-1074. [CrossRef] [PubMed]
18. Ashman, R.B.; Papadimitriou, J.M. Endothelial cell proliferation associated with lesions of murine systemic candidiasis. *Infect Immun* **1994**, *62*, 5151-5153. [Google Scholar]
19. Lee, S.C.; Li, A.; Calo, S.; Inoue, M.; Tonthat, N.K.; Bain, J.M.; Louw, J.; Shinohara, M.L.; Erwig, L.P.; Schumacher, M.A., et al. Calcineurin orchestrates dimorphic transitions, antifungal drug responses

and host-pathogen interactions of the pathogenic mucoralean fungus *Mucor circinelloides*. *Mol Microbiol* **2015**, *97*, 844-865. [CrossRef] [PubMed]

20. Wang, L.; Pittman, K.J.; Barker, J.R.; Salinas, R.E.; Stanaway, I.B.; Williams, G.D.; Carroll, R.J.; Balmat, T.; Ingham, A.; Gopalakrishnan, A.M., et al. An atlas of genetic variation linking pathogen-induced cellular traits to human disease. *Cell Host Microbe* **2018**, *24*, 308-323.e306. [CrossRef] [PubMed]
21. Barker, K.S.; Park, H.; Phan, Q.T.; Xu, L.; Homayouni, R.; Rogers, P.D.; Filler, S.G. Transcriptome profile of the vascular endothelial cell response to *Candida albicans*. *J Infect Dis* **2008**, *198*, 193-202. [CrossRef] [PubMed]
22. Uppuluri, P.; Pierce, C.G.; Thomas, D.P.; Bubeck, S.S.; Saville, S.P.; Lopez-Ribot, J.L. The transcriptional regulator Nrg1p controls *Candida albicans* biofilm formation and dispersion. *Eukaryot Cell* **2010**, *9*, 1531-1537. [CrossRef] [PubMed]
23. Romo, J.A.; Pierce, C.G.; Chaturvedi, A.K.; Lazzell, A.L.; McHardy, S.F.; Saville, S.P.; Lopez-Ribot, J.L. Development of anti-virulence approaches for candidiasis via a novel series of small-molecule inhibitors of *candida albicans* filamentation. *MBio* **2017**, *8*. [CrossRef] [PubMed]
24. Gillum, A.M.; Tsay, E.Y.; Kirsch, D.R. Isolation of the *Candida albicans* gene for orotidine-5'-phosphate decarboxylase by complementation of *S. cerevisiae* ura3 and *E. coli* pyrF mutations. *Mol Gen Genet* **1984**, *198*, 179-182. [PubMed]
25. Ramage, G.; VandeWalle, K.; Lopez-Ribot, J.L.; Wickes, B.L. The filamentation pathway controlled by the Efg1 regulator protein is required for normal biofilm formation and development in *Candida albicans*. *FEMS Microbiol Lett* **2002**, *214*, 95-100. [PubMed]
26. Saville, S.P.; Lazzell, A.L.; Monteagudo, C.; Lopez-Ribot, J.L. Engineered control of cell morphology in vivo reveals distinct roles for yeast and filamentous forms of *Candida albicans* during infection. *Eukaryot Cell* **2003**, *2*, 1053-1060. [CrossRef] [PubMed]
27. Noble, S.M.; Johnson, A.D. Strains and strategies for large-scale gene deletion studies of the diploid human fungal pathogen *Candida albicans*. *Eukaryot Cell* **2005**, *4*, 298-309. [PubMed]
28. Cleary, I.A.; Reinhard, S.M.; Miller, C.L.; Murdoch, C.; Thornhill, M.H.; Lazzell, A.L.; Monteagudo, C.; Thomas, D.P.; Saville, S.P. *Candida albicans* adhesin Als3p is dispensable for virulence in the mouse model of disseminated candidiasis. *Microbiol* **2011**, *157*, 1806-1815. [CrossRef] [PubMed]
29. Homann, O.R.; Dea, J.; Noble, S.M.; Johnson, A.D. A phenotypic profile of the *Candida albicans* regulatory network. *PLoS Genet* **2009**, *5*, e1000783. [CrossRef] [PubMed]
30. Moyes, D.L.; Wilson, D.; Richardson, J.P.; Mogavero, S.; Tang, S.X.; Wernecke, J.; Höfs, S.; Gratacap, R.L.; Robbins, J.; Runglall, M., et al. Candidalysin is a fungal peptide toxin critical for mucosal infection. *Nature* **2016**, *532*, 64. [CrossRef] [PubMed]
31. Lermann, U.; Morschhauser, J. Secreted aspartic proteases are not required for invasion of reconstituted human epithelia by *Candida albicans*. *Microbiol* **2008**, *154*, 3281-3295. [CrossRef] [PubMed]
32. Quintin, J.; Voigt, J.; van der Voort, R.; Jacobsen, I.D.; Verschueren, I.; Hube, B.; Giamarellos-Bourboulis, E.J.; van der Meer, J.W.; Joosten, L.A.; Kurzai, O., et al. Differential role of NK cells against *Candida albicans* infection in immunocompetent or immunocompromised mice. *Eur J Immunol* **2014**, *44*, 2405-2414. [CrossRef] [PubMed]
33. Liu, Y.; Filler, S.G. *Candida albicans* Als3, a multifunctional adhesin and invasin. *Eukaryot Cell* **2011**, *10*, 168-173. [CrossRef] [PubMed]

34. Nobile, C.J.; Andes, D.R.; Nett, J.E.; Smith, F.J., Jr.; Yue, F.; Phan, Q.-T.; Edwards, J.E., Jr.; Filler, S.G.; Mitchell, A.P. Critical role of Bcr1-dependent adhesins in *C. albicans* biofilm formation *in vitro* and *in vivo*. *PLoS Pathog* **2006**, *2*, e63. [CrossRef] [PubMed]
35. Grubb, S.E.; Murdoch, C.; Sudbery, P.E.; Saville, S.P.; Lopez-Ribot, J.L.; Thornhill, M.H. *Candida albicans*-endothelial cell interactions: a key step in the pathogenesis of systemic candidiasis. *Infect Immun* **2008**, *76*, 4370-4377. [CrossRef] [PubMed]
36. Desai, J.V. *Candida albicans* Hyphae: From Growth Initiation to Invasion. *J Fungi (Basel)* **2018**, *4*. [CrossRef] [PubMed]
37. Jacobsen, I.D.; Wilson, D.; Wächtler, B.; Brunke, S.; Naglik, J.R.; Hube, B. *Candida albicans* dimorphism as a therapeutic target. *Expert Rev Anti Infect Ther* **2012**, *10*, 85-93. [CrossRef] [PubMed]
38. Gow, N.A.; van de Veerdonk, F.L.; Brown, A.J.; Netea, M.G. *Candida albicans* morphogenesis and host defence: discriminating invasion from colonization. *Nat Rev Microbiol* **2011**, *10*, 112-122. [CrossRef] [PubMed]
39. da Silva Dantas, A.; Lee, K.K.; Raziunaite, I.; Schaefer, K.; Wagener, J.; Yadav, B.; Gow, N.A. Cell biology of *Candida albicans*-host interactions. *Curr Opin Microbiol* **2016**, *34*, 111-118. [CrossRef] [PubMed]
40. Cao, Y.; Gong, Y.; Liu, L.; Zhou, Y.; Fang, X.; Zhang, C.; Li, Y.; Li, J. The use of human umbilical vein endothelial cells (HUVECs) as an in vitro model to assess the toxicity of nanoparticles to endothelium: a review. *J Appl Toxicol* **2017**, *37*, 1359-1369. [CrossRef] [PubMed]
41. Antoine, M.; Wirz, W.; Tag, C.G.; Mavituna, M.; Emans, N.; Korff, T.; Stoldt, V.; Gressner, A.M.; Kiefer, P. Expression pattern of fibroblast growth factors (FGFs), their receptors and antagonists in primary endothelial cells and vascular smooth muscle cells. *Growth Factors* **2005**, *23*, 87-95. [CrossRef] [PubMed]
42. Müller, V.; Viemann, D.; Schmidt, M.; Endres, N.; Ludwig, S.; Leverkus, M.; Roth, J.; Goebeler, M. *Candida albicans* triggers activation of distinct signaling pathways to establish a proinflammatory gene expression program in primary human endothelial cells. *J Immunol* **2007**, *179*, 8435. [CrossRef] [PubMed]
43. Wilson, D.; Mayer, F.L.; Miramon, P.; Citiulo, F.; Slesiona, S.; Jacobsen, I.D.; Hube, B. Distinct roles of *Candida albicans*-specific genes in host-pathogen interactions. *Eukaryot Cell* **2014**, *13*, 977-989. [CrossRef] [PubMed]
44. Orozco, A.S.; Zhou, X.; Filler, S.G. Mechanisms of the proinflammatory response of endothelial cells to *Candida albicans* infection. *Infect Immun* **2000**, *68*, 1134-1141. [CrossRef] [PubMed]
45. Phan, Q.T.; Belanger, P.H.; Filler, S.G. Role of hyphal formation in interactions of *Candida albicans* with endothelial cells. *Infect Immun* **2000**, *68*, 3485-3490. [CrossRef] [PubMed]
46. Gow, N.A.; Netea, M.G.; Munro, C.A.; Ferwerda, G.; Bates, S.; Mora-Montes, H.M.; Walker, L.; Jansen, T.; Jacobs, L.; Tsoni, V., et al. Immune recognition of *Candida albicans* beta-glucan by dectin-1. *J Infect Dis* **2007**, *196*, 1565-1571. [CrossRef] [PubMed]
47. Rowen, J.L.; Tate, J.M. Heat-Killed *Candida albicans* elicit more interleukin 8 (IL-8) from human monocytes than live yeast. *Pediatr Res* **1998**, *43*, 156.
48. van de Veerdonk, F.L.; Marijnissen, R.J.; Kullberg, B.J.; Koenen, H.J.P.M.; Cheng, S.-C.; Joosten, I.; van den Berg, W.B.; Williams, D.L.; van der Meer, J.W.M.; Joosten, L.A.B., et al. The macrophage mannose receptor induces IL-17 in Response to *Candida albicans*. *Cell Host Microbe* **2009**, *5*, 329-340. [CrossRef] [PubMed]

49. Kumar, R.; Saraswat, D.; Tati, S.; Edgerton, M. Novel aggregation properties of *Candida albicans* secreted aspartyl proteinase Sap6 mediate virulence in oral Candidiasis. *Infect Immun* **2015**, *83*, 2614-2626. [CrossRef] [PubMed]
50. Richardson, J.P.; Willems, H.M.E.; Moyes, D.L.; Shoaie, S.; Barker, K.S.; Tan, S.L.; Palmer, G.E.; Hube, B.; Naglik, J.R.; Peters, B.M. Candidalysin drives epithelial signaling, neutrophil recruitment, and immunopathology at the vaginal mucosa. *Infect Immun* **2017**, 10.1128/iai.00645-17. [CrossRef] [PubMed]
51. Wilson, D.; Naglik, J.R.; Hube, B. The missing link between *Candida albicans* hyphal morphogenesis and host cell damage. *PLoS Pathog* **2016**, *12*, e1005867. [CrossRef] [PubMed]
52. Ben-Ami, R.; Lewis, R.E.; Leventakos, K.; Kontoyiannis, D.P. *Aspergillus fumigatus* inhibits angiogenesis through the production of gliotoxin and other secondary metabolites. *Blood* **2009**, *114*, 5393-5399. [CrossRef] [PubMed]
53. Richardson, J.P.; Mogavero, S.; Moyes, D.L.; Blagojevic, M.; Krüger, T.; Verma, A.H.; Coleman, B.M.; De La Cruz Diaz, J.; Schulz, D.; Ponde, N.O., et al. Processing of *Candida albicans* Ece1p is critical for candidalysin maturation and fungal vrrirulence. *MBio* **2018**, *9*, e02178-02117. [CrossRef] [PubMed]
54. Ganz, T. Angiogenin: an antimicrobial ribonuclease. *Nat Immunol* **2003**, *4*, 213. [PubMed]
55. Datta, M.; Via, L.E.; Kamoun, W.S.; Liu, C.; Chen, W.; Seano, G.; Weiner, D.M.; Schimel, D.; England, K.; Martin, J.D., et al. Anti-vascular endothelial growth factor treatment normalizes tuberculosis granuloma vasculature and improves small molecule delivery. *Proc Natl Acad Sci* **2015**, *112*, 1827-1832. [CrossRef] [PubMed]
56. Oehlers, S.H.; Cronan, M.R.; Scott, N.R.; Thomas, M.I.; Okuda, K.S.; Walton, E.M.; Beerman, R.W.; Crosier, P.S.; Tobin, D.M. Interception of host angiogenic signalling limits mycobacterial growth. *Nature* **2015**, *517*, 612-615. [CrossRef] [PubMed]
57. Polena, H.; Boudou, F.; Tilleul, S.; Dubois-Colas, N.; Lecointe, C.; Rakotosamimanana, N.; Pelizzola, M.; Andriamandimby, S.F.; Raharimanga, V.; Charles, P., et al. *Mycobacterium tuberculosis* exploits the formation of new blood vessels for its dissemination. *Sci Rep* **2016**, *6*, 33162. [CrossRef] [PubMed]
58. Van De Veerdonk, F.L.; Netea, M.G.; Joosten, L.A.; Van Der Meer, J.W.M.; Kullberg, B.J. Novel strategies for the prevention and treatment of *Candida* infections: the potential of immunotherapy. *FEMS Microbiol Rev* **2010**, *34*, 1063-1075. [CrossRef] [PubMed]