Kinetics of Titan cells generation and transcriptome modifications comparing three in vitro protocols

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Objectives: To face and escape his environment and the host immune response, Candida neoformans is able to change his morphology (Titan cells) and his metabolism (dormancy, quiescence). Titan cells (TC) have been observed in lungs and brains in experimental mouse models of cryptococcosis and in patients. In 2018, three independent teams published three protocols allowing the reproducible generation of TC in vitro [Hommel B et al., (AA); Trevijano-Contador N, et al., (OZ), and Dambuza DM et al., (EB), in PLoS Pathogens 2018]. TC generated in these protocols exhibited the same features as the TC described in vivo. Our objective was to compare and describe the three protocols in parallel to highlight common and different features that can impact further study using those specific protocols.

Methods: A total of 22 h before starting the kinetics of the TC generation, the three protocols requested a pre-culture at 30°C in three different liquid media. The medium for TC production for the three labs was also different but a common factor is the addition of fetal calf serum (FBS) for OZ and EB. This kinetics was evaluated for size and quantity (% of TC produced over a 72 h period (H0, H18, H24, H48, H72) at 30°C under shaking for AA while OZ and EB protocols incubate the cells at 37°C and 5% of CO₂, while the whole transcriptome was analyzed at H0, H3, H7, and H18 in triplicates.

Results: OZ generated the highest percentage of TC, 63.1% and 58.2% at H18 and H24, and decreased drastically down to 6.7% at H48; EB reached a high percentage of TC at H24 for 46.7% and dropped <10% until the end of the kinetics. AA did not reach a quantity of TC as high as the two other protocols but it remained constant over a period of H72 (Table 1). RNA sequencing preliminary analysis showed some differences in genes expressed at the different time points analyzed. The

Table 1.

| Compounds | Binding energy (Kcal/mol) | No. of Hydrogen bonds |
|-----------|--------------------------|-----------------------|
| α-defensin 5 like peptide | -22.07 | 1 |
| M1        | -54.47                   | 4 |
| M2        | -49.46                   | 2 |
| M3        | -45.34                   | 1 |
| M4        | -48.62                   | 2 |
| M5        | -43.24                   | 1 |
| M6        | -40.65                   | 1 |

Table 2.
PCA analysis revealed that the replicates of each protocol for the 4-time points analyzed are closed to each other, related to the good quality of our experiment. The differential gene expression (DGE) showed significant ($P < .01$ and Log2 fold change $>1$) differences at H8 which highlights the impact of the protocols on the TC process. The highest number of DGE were observed between H0 and H7 for the three protocols, where about two 410 DGE, two 100 DGE, and two 50 AA, OZ, and EB, respectively. After analysis of the PCA plot during the kinetics, EB and OZ are grouped while AA is not. That could be explained by the presence of FBS in OZ and EB protocols.

Conclusion: By running the three protocols in parallel, we showed here that the kinetics of TC generation differ between each other with a significant variation of the transcriptome. This is an important finding that gives the way to compare more deeply the transcriptome of C. neoformans during TC generation with the final goal is to identify the genes associated with TC generation.

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Scalp fungal microbiome and sebum composition in males with and without androgenetic alopecia

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Objectives: Lipophilic Malassezia species are abundant in the scalp microbiome; we investigated the scalp microbiome and sebum composition of patients with androgenetic alopecia (AGA) and aimed to identify factors accelerating AGA progression.

Materials and Methods: Scalp scale samples (sebum) were collected from 35 male Japanese patients with AGA and 35 healthy individuals. Fungal RNA genes were amplified by PCR and the amplicons were sequenced on the MiSeq platform. The extent of fungal colonization was determined by qPCR. We used gas chromatography/mass spectrometry to measure the sebum levels of free fatty acids, dihydrocerics, triglycerides, squalene, free cholesterol, cholesteryl esters, and wax.

Results and Discussion: Malassezia verticillata predominated in all AGA (64.7%) and non-AGA age groups (44.6%). qPCR revealed that Malassezia colonization was more intense in the AGA than non-AGA group, regardless of age; the Malassezia level was significantly higher in AGA subjects aged 10–59 than 60–69 years. The TG level was significantly higher in the AGA than non-AGA group ($P < .05$), but the free fatty acid, squalene, and free cholesteryl levels were significantly lower ($P < .01$).

Conclusion: Thus, the scalp fungal microbiome and sebum composition may influence AGA development.

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‘It’s not Fungus, its Nocardia’ an elementary diagnostic challenge for draining sinus on abdominal wall (rare): a case report

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Introduction: A rare finding of abdominal wall clinical presentation of persistent progressive inflammation with draining sinus with no granules caused by Nocardia brasiliensis.

Methods: History: A 22-year-old presented to the Dermatology OPD with complaints of swelling and tenderness and discharging sinuses with no granules around the umbilical region in the lower abdominal area for three years. His initial fine needle aspiration cytology specimen report was inconclusive. He received anti-tuberculosis treatment based on a positive Montoux test and family history from outside the hospital.

Initially punch biopsy samples were sent for fungal processing to our laboratory which was inconclusive. Report pus aspirate and punch biopsy samples were subjected to conventional techniques. The sample was inoculated on Sabouraud’s Dextrose agar, Brain heart Infusion agar, and Lowenstein-Jensen media. Direct Smear was subjected to Gram stain and Modified Ziehl Neelsen stain with 1% Sulfuric acid as decolorizer.

Results:
1. On Gram stain, Gram-positive filamentous bacilli against a background of pus cells in pus aspirate only (not in punch biopsy specimen).
2. Modified Ziehl Neelsen stain with 1% Sulfuric acid decolorizer was performed on all three samples. Beaded acid-fast filamentous bacilli with plenty of pus cells in the background were seen in pus aspirate only (not in punch biopsy specimen).
3. No fungal elements were observed on the 20% KOH mount.
4. Clinician were notified immediately with the provisional report of possible Actinomycetoma due to Nocardia sp.
5. Growth was observed within 9 days on SDA as well as LJ. It was a chalky white, dry colony to begin with that turned straw-colored yellow in another week’s time. Smear from the colony showed Gram-positive filamentous bacilli which in Modified ZN Smear were acid-fast filamentous beaded bacilli. The isolate was identified as Nocardia species. This was further confirmed as Nocardia brasiliensis by MALDI-TOF.
6. On admission, the patient was initially started on Ibu, Amikacin and then changed to Modified Ramane regimen of double dose Gentamicin and Ceftriaxone. His lesions started showing improvement over 2 weeks of in-patient treatment. He was discharged on oral treatment thereafter.

Conclusion:
1. Abdominal wall clinical presentation of persistent progressive inflammation with draining sinus with no granules caused by N. brasiliensis is a rare clinical entity in Mycosarcoma. The differential diagnosis would lead to bacterial or fungal etiology or neoplasia.
2. Delay in correct diagnosis led to the chronality of the clinical presentation with inappropriate therapy.
3. For a chronic destructive debilitating infective mycosarcoma presentation, appropriate microbiological diagnosis become essential to have early correct diagnosis with proper sampling technique to guide the appropriate therapy as per the causative pathogen.