In vivo analysis of *A. fumigatus* sulphur-related transcriptome during infection using a NanoString nCounter Platform

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The regulation of sulphur assimilation has been demonstrated to be essential for *A. fumigatus* virulence (1). Therefore, sulphur metabolism is a promising pathway to identify relevant virulence traits which can be exploited as targets for fighting fungal infection. To identify gene products which are relevant for infection one approach is to analyse the *in vivo* transcriptome. However, detection and analysis of the fungal transcriptome during infection is a persistent challenge, as fungal RNA usually represents between 0.05 and 0.5% of the total RNA isolated from infected organs (2). The aim of this work is to determine specific sulphur metabolic related genes which are relevant for infection of mammalian hosts using the NanoString nCounter Platform, whose high sensitivity allows it to detect as little as 0.5 fM of mRNA.

1. Murine lung constitutes a sulphur limiting environment

For *in vitro* samples, an overnight *A. fumigatus* mycelium was shifted to minimal media with different sulphur sources, incubated for two hours and RNA isolated. *In vivo* RNAs were isolated from the lungs of leukopenic mice infected with 2×10^5 conidia of *A. fumigatus* and culled three days post-infection. The expression of 51 sulphur-related genes on the *in vitro* samples have generated clear and distinct profiles which can be clustered to show the similarity among different concentrations and sources tested. Comparison and hierarchical clustering with the *in vivo* samples suggests that the murine lung constitutes a sulphur limiting environment, with some differential characteristics.

3. The exploited S-sources found in the lung seem to be organic

The analysis of the expression pattern of sulphur-related genes obtained from 2 independent *in vivo* samples were compared to each *in vitro* condition tested. Among the most up-regulated genes, several glutathione transferases, such as AFUA_4G01440 and AFUA_6G00760, or the Cystathionine-γ-lyase mecB (AFUA_8G04340) could be identified as the most up-regulated genes during the infection. Furthermore, this analysis suggests that the exploited S-sources in lungs might be organic.

2. Iron metabolism showed the expected expression during the infection

The analysis of genes involved in iron metabolism showed the expected expression when 2 independent *in vivo* samples were compared to *in vitro* conditions. Whereas *sreA* appeared up-regulated in *in vitro* conditions, *hapX* and siderophore related genes (*mirB* and *sidA*) showed up-regulation during the infection.

4. MTE protocol allows analysing samples in which the expected amount of RNA is below the detection limit

Direct comparison between the fold change (FC) of amplified and non-amplified *in vitro* (-S limiting conditions) and *in vivo* samples (lungs extracted from 2 different mice) have demonstrated that the Multiplex Target Enrichment (MTE) amplification is linear and maintain the rates. This will become relevant for the analysis of samples in which the expected amount of RNA is below the detection limit (e.g. patient samples).

CONCLUSIONS

1. NanoString Technology is a promising approach to analyse the transcription levels during fungal infection.
2. Murine lung constitutes a sulphur limiting environment in which the exploited S-sources seem to be organic.

Future work

1. To better determine the status of sulphur-related metabolism *in vivo*, gene expression will be analysed at different infection times, in different immunosuppression models (leukopenic and cortisone) and using a conditional *metR* mutant (master sulphur transcriptional factor).
2. Gene expression will be assessed in patient samples.
3. Promising genes, with high and consistent up-regulation *in vivo*, will be investigated further.

REFERENCES

1. Amich *et al.*, 2013 PLoS Pathog.
2. Xu *et al.*, 2016 Methods Mol Biol