27th ECCMID Vienna, Austria 22 – 25 April 2017

The congress of ESCMID

Session: OS185 Immunity and immunogenetics of infections in immunocompromised hosts

Category: 10a. Host genetics: infection susceptibility & immunodeficiency

25 April 2017, 12:06 - 12:16
OS0902

Transcriptomic regulatory analysis of the dendritic cell response to post-lung transplant invasive aspergillosis

Amit Adlakha*[1], Darius Armstrong-James[2], Boris Lenhard[3]

[1]*Imperial College; Computational Regulatory Genomics*

[2]*National Heart & Lung Institute*

[3]*Mrc Clinical Sciences Centre*

Background: Lung transplant recipients on calcineurin inhibitors are susceptible to have increased susceptibility to invasive aspergillosis. Tacrolimus (FK506) diminishes the innate immune response to *Aspergillus fumigatus* infection partly by inhibition of the calcineurin-NFAT axis. We investigated the effects of FK506 on transcriptional regulation in dendritic cells (DC's), and assessed interferon-gamma as a possible treatment, with a combination of RNA-Seq and histone modification ChIP-seq.

Material/methods: Healthy volunteer monocytes were negatively isolated from gradient-centrifugation-selected PBMC's and differentiated into DC's with GM-CSF and IL-4. DC's were treated with FK506, interferon-gamma and/or inoculated with swollen conidia of *A.fumigatus.* For RNA-Seq, extracted mRNA was poly-A purified and reverse-transcribed to ds-DNA, and for ChIP-seq, DNA was cross-linked, sonicated, then immunoprecipitated with antibodies against histone marks H3k4me1 and H3k27ac. Resultant DNA was PCR-amplified to generate libraries for next generation sequencing on the Illumina HiSeq 2500. Computational sequencing analysis pipelines used open-source C++ and R-based packages (Bowtie, Kallisto, edgeR and MACS).

Results: *A.fumigatus* infection in DC's elicited upregulation of genes belonging to two key groups of early-phase response transcription factors – the early growth response family (EGR1 - log fold-change 4.90, FDR p-value=0.0003) and the nuclear receptor family (NR4A2- logFC 6.96, $p=1.56x10^{-6}$). FK506 treatment ablated significant differential expression of these genes whilst subsequent interferon-gamma treatment restored their upregulation (EGR1 - logFC 4.43, p=0. 00093; NR4A2 - logFC 5.56, p=0. 00034).

Active gene enhancers regions were identified by presence of significant peaks of H3k4me1 and H3k27ac antibody binding. Motif analysis of enhancers within regulatory domains around differentially-expressed genes identified enrichment of core binding motifs of NFAT ($p=7.8 \times 10^{-9}$) and FOXF2 ($p=8.6 \times 10^{-10}$) transcription factors, which was lost after FK506 treatment.

Conclusions: *A.fumigatus* infection in DC's elicited upregulation of genes belonging to two key groups of early-phase response transcription factors – the early growth response family (EGR1 - log fold-change 4.90, FDR p-value=0.0003) and the nuclear receptor family (NR4A2- logFC 6.96, $p=1.56 \times 10^{-6}$). FK506 treatment ablated significant differential expression of these genes whilst subsequent interferon-gamma treatment restored their upregulation (EGR1 - logFC 4.43, p=0. 00093; NR4A2 - logFC 5.56, p=0. 00034).

Active gene enhancers regions were identified by presence of significant peaks of H3k4me1 and H3k27ac antibody binding. Motif analysis of enhancers within regulatory domains around differentially-expressed genes identified enrichment of core binding motifs of NFAT ($p=7.8 \times 10^{-9}$) and FOXF2 ($p=8.6 \times 10^{-10}$) transcription factors, which was lost after FK506 treatment.