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Title: Stable isotope labelled amino acid culturing: A novel approach to identify pathways involved in MIF signalling

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Body: Chronic obstructive pulmonary disease (COPD) shows poor clinical improvement with steroids. Macrophage Migration Inhibitory Factor (MIF) counter-regulates steroid action although the mechanism is unknown. Stable isotope labelled amino acid cell culture (SILAC) involves incorporating differently labelled amino acids into cells to allow comparison of protein profiles. SILAC was used to investigate the effect of MIF on intracellular signalling pathways. THP-1 cells were grown in SILAC media and then stimulated with or without MIF for 24 hours, the cells were lysed and proteins extracted. Incorporation of the labelled amino acids were measured and analysed by LC-MSMS. Pathways identified were validated by qRT-PCR, ELISA and Western blotting. MIF treatment up-regulated 70 and down-regulated 163 proteins; pathway analysis identified the RIG-I-like receptor (RLR) pathway. In THP-1 cells, as the main MIF target, MIF was induced by Poly I:C in a concentration-dependent manner and RV16 infection induced MIF expression in a time-dependent manner. BAL macrophage proteins were analysed at baseline and 7 days post RV16 infection in non-smokers, smokers and COPD patients. Smokers and COPD patients showed a significant decrease in intracellular MIF post-infection. We used proteomics to define a novel mechanism of MIF action in THP-1 cells and translated this to primary human cells. Viral activation induced MIF release from THP-1 cells and was associated with a loss of intracellular MIF in BAL macrophages 7 days post infection in smokers and COPD patients. This proteomics approach has the ability to define novel mechanisms for understanding drug or disease effects.