

## **Identification of pro-fibrotic macrophages populations by single-cell transcriptomic analysis in West Highland white terriers affected with canine idiopathic pulmonary fibrosis.**

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Canine idiopathic pulmonary fibrosis (CIPF) is a not well understand disease which affects old West Highland white terriers (WHWTs) and mimics idiopathic pulmonary fibrosis (IPF) in man. Recent studies in IPF using the single-cell RNA sequencing (scRNA-seq) technique revealed the presence of profibrotic macrophages populations in the lung. Here we used the scRNA-seq to characterize disease-related heterogeneity within cell subsets of macrophages/monocytes (Ma/Mo) in the BALF of 5 WHWTs affected with CIPF in comparison with 3 healthy WHWTs. Five subsets of Ma/Mo were identified. Among them, a monocytes subset present in larger proportion in CIPF WHWTs showed a gene expression profile enriched for pulmonary fibrosis processes (PFPs) (normalized enrichment score (NES) = 1.85,  $q$ -value = 0.002). Eight genes associated with PFPs were significantly overexpressed in this subset including *CCL2*, *SPP1*, *FN1*, *CCL3*, *TIMP1*, *IL1RN*, *CXCL8* and *CCL4*. A monocytes-derived macrophages subset enriched for PFPs (NES = 1.87,  $q$ -value = 0.007) was also identified with differentially expressed genes between CIPF and healthy WHWTs. Expression in CIPF dogs in this subset was enriched for PFPs (NES = 2.01,  $q$ -value = 0.008) with significant overexpression of 4 genes associated with PFPs including *FN1*, *SPP1*, *CXCL8* and *PLAU*. ScRNA-seq analysis of BALF specimens from healthy and CIPF WHWTs identified pro-fibrotic Ma/Mo populations enriched in pro-fibrotic genes suggesting the implication of these subpopulations in CIPF processes. Overexpressed molecules were also identified that could be used as biomarkers and/or therapeutic targets in the future.