

1 **Single-cell RNA-Seq reveals a repair pattern in cystic lesions** 2 **in steroid induced osteonecrosis of the femoral head**

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16 **Abstract**

17 **Background:** Studies have indicated that cystic lesions play a crucial role in the
18 repair processes of steroid-induced osteonecrosis of the femoral head and its
19 subsequent collapse. Here, we employed single-cell RNA sequencing (scRNA-seq)
20 technology to investigate the transcriptomic landscape and repair mechanisms of
21 cystic lesions in SIONFH.

22 **Methods:** We applied scRNA-seq combined with computational approaches to
23 characterize distinct cell subsets and their molecular signatures within cystic lesions
24 from three SIONFH patients. Additionally, histological assays were conducted to
25 observe pathological manifestations of these lesions.

26 **Results:** Eight cell types were identified in cystic lesions of SIONFH. Among them,
27 chondrocytes were divided into five subgroups. Among them, chondrocytes were
28 divided into five subgroups: homeostatic chondrocytes (HomC), fibrocartilage
29 chondrocytes (FC), prehypertrophic chondrocytes (preHTC), inflammatory
30 chondrocytes (InflamC), and hypertrophic chondrocytes (HTC). Additionally,
31 histological assays showed the presence of chondrocytes and a transition zone from
32 chondrocytes to bone tissue within the cystic lesions. Notably, we report that one of
33 the HTC clusters with CLIC3⁺ expression exhibited a strong involvement in bone
34 mineralization, osteoblast differentiation, and cell differentiation.

35 **Conclusion:** We have delineated the cellular heterogeneity and molecular signatures
36 of cystic lesions in SIONFH. The results reveal a distinct repair program within these
37 lesions, which might be driven by chondrocyte hypertrophy and might culminate in
38 osteogenic differentiation.

39 **Keywords:** steroid-induced osteonecrosis of the femoral head; single-cell RNA
40 sequencing; chondrocyte; cystic lesions; osteogenic differentiation