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Macrophage-lineage Transcriptome Networking Reveals SMAD3 as a Novel Regulator for CAF Formation in NSCLC

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INTRODUCTION

Cancer-associated fibroblasts (CAF) are important for promoting cancer progression. Their origins are highly heterogeneous and still largely unclear, better understanding would uncover novel therapeutic target for cancer. Recently, we unexpectedly revealed macrophages can further transit into myofibroblast in diseased kidney, implying its potential in CAF generation. Here, we successfully resolved the transcriptome dynamics of tumour microenvironment (TME) at single-cell resolution, therefore discovering Smad3 of macrophage as a novel therapeutic target for blocking CAF formation in NSCLC.

METHOD

Presence of CD68⁺CAF transiting cells was evaluated on the cancer patient biopsies. Fate mapping study examined the development of CD68⁺CAF in a syngeneic mouse lung carcinoma model LLC with 10X scRNA-sequencing. Pathogenic role of CD68⁺CAF was demonstrated on NOD/SCID mice with LLC-tumour. Eventually, gene network analysis identified the regulatory mechanism of CD68⁺CAF formation in NSCLC, the regulatory role of macrophage-specific Smad3 in CAF formation was confirmed in LLC-bearing mice.

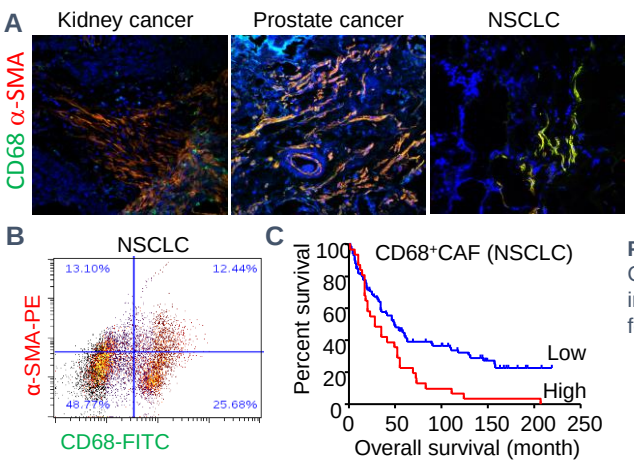

Figure 1. CD68⁺CAF in human TME. (**A**) Confocal imaging evidences CD68⁺CAF in human cancers. (**B**) Flow analysis shows that CD68⁺ CAF is a rich source CAF in NSCLC, (**C**) its abundancy associated with patient mortality (N=120).

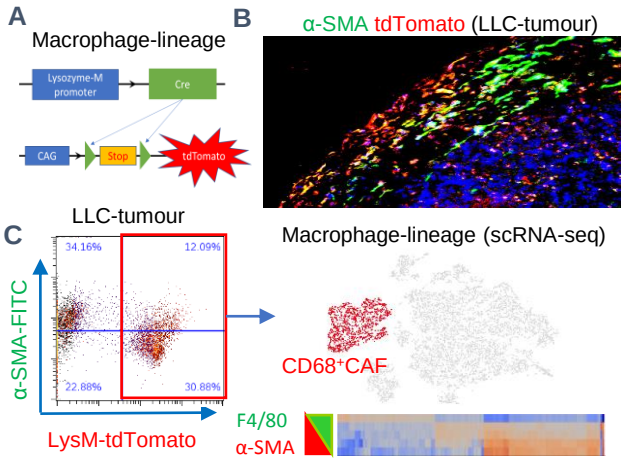

Figure 3. Fate mapping study of CD68⁺CAF in LLC model. (**A**) A system allows macrophage-lineage cells permanently expressing tdTomato *in vivo*. Thus, macrophage-derived CAF in LLC-tumour are detected at both protein and transcriptome levels by (**B**) confocal imaging and (**C**) 10X scRNA-seq (N=6).

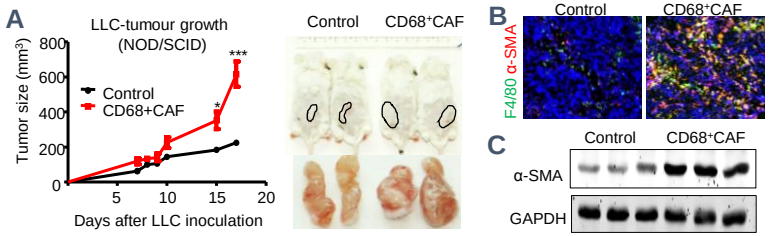

Figure 2. Adoptive transfer of CD68⁺CAF largely promotes cancer progression. CD68⁺CAF generated from bone marrow derived macrophages (BMDM) *in vitro* are inoculated into LLC-bearing mice, which largely promotes (**A**) growth and (**B**) CAF formation of the tumour *in vivo* (n=6).

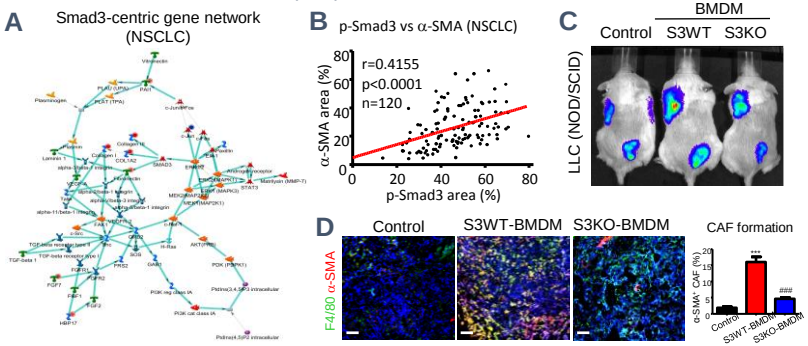

Figure 4. Smad3 is a novel regulator for CD68+CAF formation. (**A**) MetaCore analysis reveals a Smad3-centric gene network from CD68⁺CAF in NSCLC. (**B**) Our cohort detects a strong correlation between Smad3 activation and CAF formation in NSCLC (N=120).**C, D**, BMDM markedly enhances tumour growth and CAF formation, which are effectively blocked by the genetic silencing of Smad3 (S3KO) (*in vivo* n=6).

Conclusion

It is the first study intensively elucidated the protumoral role and underlying mechanism of CD68⁺CAF formation. We discovered macrophage as the major source of CD68⁺ CAF at single-cell resolution. Finally, we identified Smad3 of macrophage as a novel therapeutic target for blocking the CD68⁺ CAF formation in NSCLC.

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