FAP+ stromal cells are necessary and sufficient to maintain marrow homeostasis

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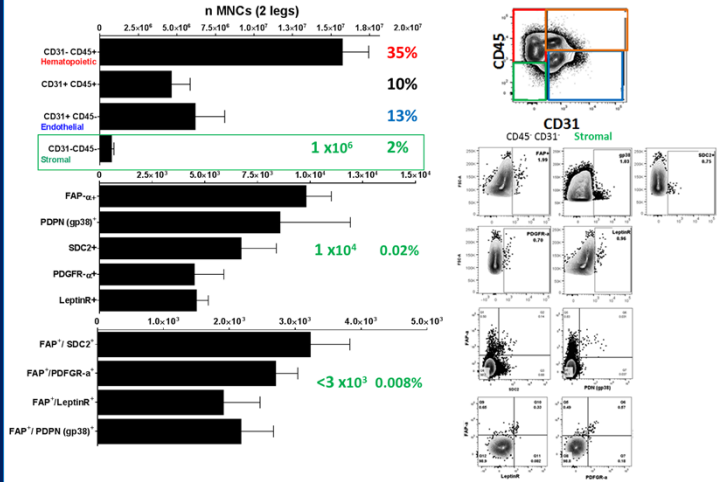
Background

A recent Phase 3 clinical trial has demonstrated that Mesenchymal stromal cell (MSC) therapies[1] can ameliorate immune-mediated inflammatory diseases, however the endogenous role of stromal cells are only beginning to be elucidated. Fibroblast Activation Protein-α (FAP) is a marker of tissue resident stromal cells (SCs) removable by FAP^{DM2}-DTR system. FAP$^+$ cell ablation induces a pathogenic phenotype that is alleviated by Stromal cell (SC) administration.

1. FAP-α is expressed in endogenous stromal cells

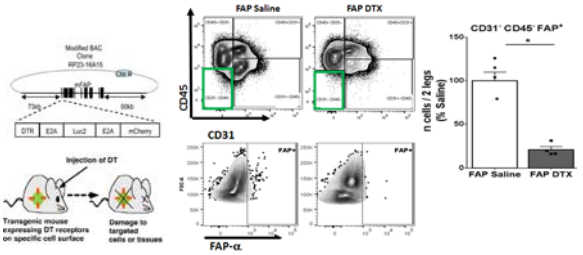
The stromal cell compartment in bone marrow (BM) (2%) contains up to 0.02% of stromal cell characterized by the expression of FAP-α, PDPN, SDC2, PDGFR-α or Leptin-R. FAP-α$^+$ stromal cell co-localized with other stromal cells markers are rare cell populations.



Digested BM tissue, Mononuclear cells (MNCs) stained and analysed by Flow cytometry to show the percentage of stromal cells within the BM

2. Conditional ablation of FAP+ Stromal cells

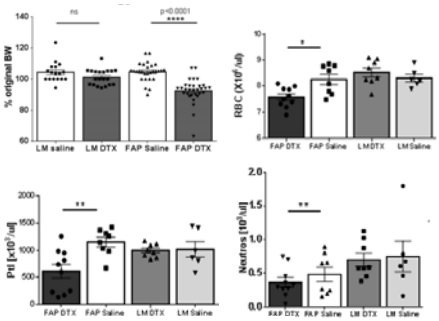
FAP expressing cells were ablated by administration of two doses of Diphtheria toxin (DTX) in the FAP^{DM2} strain[2,3].



Representative flow cytometry dot plots show FAP deletion in CD45/Ter119- CD31- stromal cell population. Bar graph shows the percentage of ablation.

3. FAPα+ cell ablation causes weight loss, Anaemia and Neutropenia

Body weight at day 3 after second boost of DTX is reduced in FAP-DTR mice.



Anaemia is confirmed by the reduction of red blood cells (RBC).

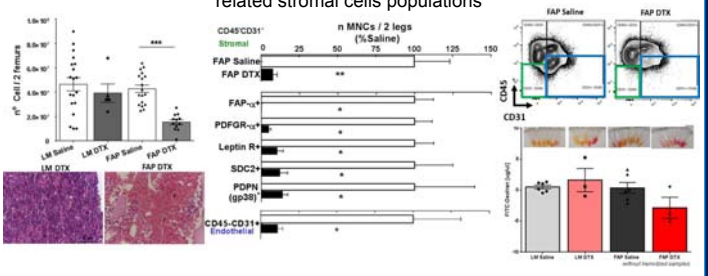
Additionally, we report thrombocytopenia and neutropenia in Peripheral blood (PB).

4. FAPα+ cell ablation leads to marrow hypo-cellularity

MNC reduction confirmed by H&E

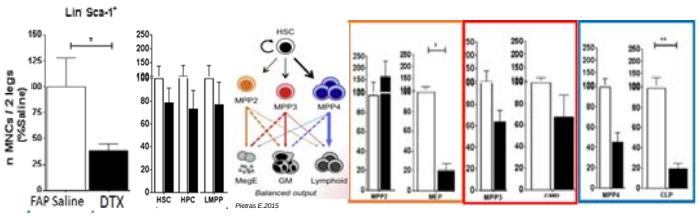
FAP+ cell ablation also depletes related stromal cells populations

Permeability in blood vasculature increased



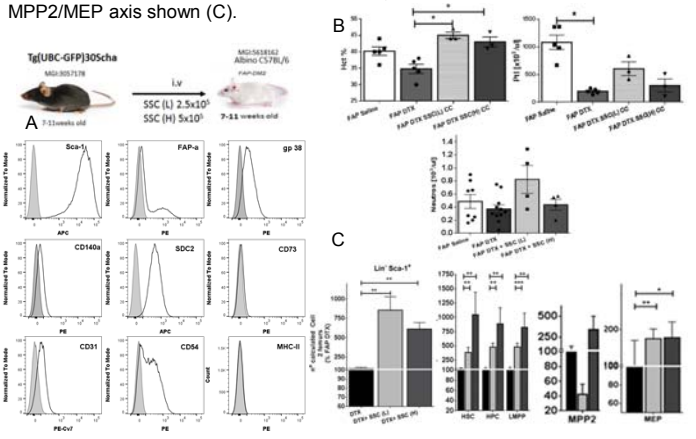
5. FAPα+ cell ablation alters haematopoiesis

The blood forming process is altered from the progenitors (Lin- Sca-1+) to the multipotent (HSC, HPC and LMPP) and oligopotent populations (MEP, CLP and GMP). FAP+cell ablation leads to increased MPP2 cell numbers, suggesting a regenerative response.



6. Intravenous administration of a single dose of syngeneic marrow stromal cells alleviates FAP ablation associated phenotypes.

A characterized stromal cell population (A) from syngeneic mouse strain increases the blood volume formed (Htc), ameliorates thrombocytopenia and neutropenia in PB (B). BM switches from regenerative to homeostasis status as MPP2/MEP axis shown (C).



Conclusions

FAPα+ SCs are necessary for BM homeostasis. A single dose of marrow SCs is sufficient to restore significantly anaemia, alleviate thrombocytopenia and neutropenia whilst increasing the progenitor's percentage in BM.

Statistical analysis: Mean and SEM values. Mann-Whitney test for 2 groups comparison and *Kruskal-Wallis test, Dunn's multiple comparisons test for 3 or more* (α=0.05). **Stromal cell expression and FAP Ablation**: FAP saline (n=6), FAP DTX (n=6). **Body Weight**: LM saline (n=17), LM DTX (n=20), FAP Saline (n=28) and FAP DTX (n=31). **Blood counts**: FAP DTX (n=10), FAP Saline (n=8), LM Saline (n-=8), LM DTX (n=6). (A) **Hypo cellularity** bar graph. LM saline (n=20), LM DTX (n=5), FAP saline (n=20) and FAP DTX (n=12). (B) FAP saline (n=6), FAP DTX (n=6). **Haematopoiesis**: FAP saline (n=6), FAP DTX (n=6). **SSC therapy:** (B) FAP DTX (n=6), FAP DTX+SSC(L) (n=8), FAP DTX+SSC(H) (n=8).

References

1.Panes J. et al. 2016 Sep 24;388(10051):1281-90. 2.Roberts et al. J Exp Med, 2013. 210(6): p. 1137-51. 3.Kraman et al. Science, 2010. 330(6005): p. 827-30.

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