Life without MSC?

An animal model based on FAP+ endogenous stromal cell ablation.

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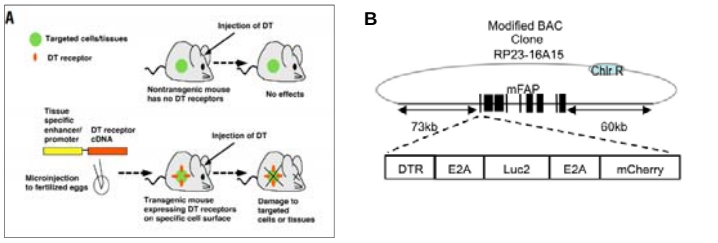
Abstract: Tissue-derived Mesenchymal Stromal Cells (MSC) comprise a mixed population of fibroblastic cells that can be isolated from bone marrow, placenta, adipose and other sources by adherence to tissue culture plastic and formation of colony forming unit-fibroblasts (CFU-F). MSC secrete potent immune-modulatory and angiogenic factors and so represent a valid therapeutic option for complicated inflammatory and ischemic diseases. Questions remain over the endogenous identity and function of MSC in vivo, as well as the heterogeneity and function of therapeutic MSC preparations in vitro. We have shown that antibodies to CD362 - a heparan sulphate proteoglycan that mediates Left-Right patterning, ECM deposition and vascular development in embryogenesis - can be used for the prospective isolation of highly enriched and therapeutically active preparations of MSC from multiple species. Fibroblast activation protein (FAP) is a marker of tissue and tumour-resident stromal cells that are necessary for marrow and muscle homeostasis.

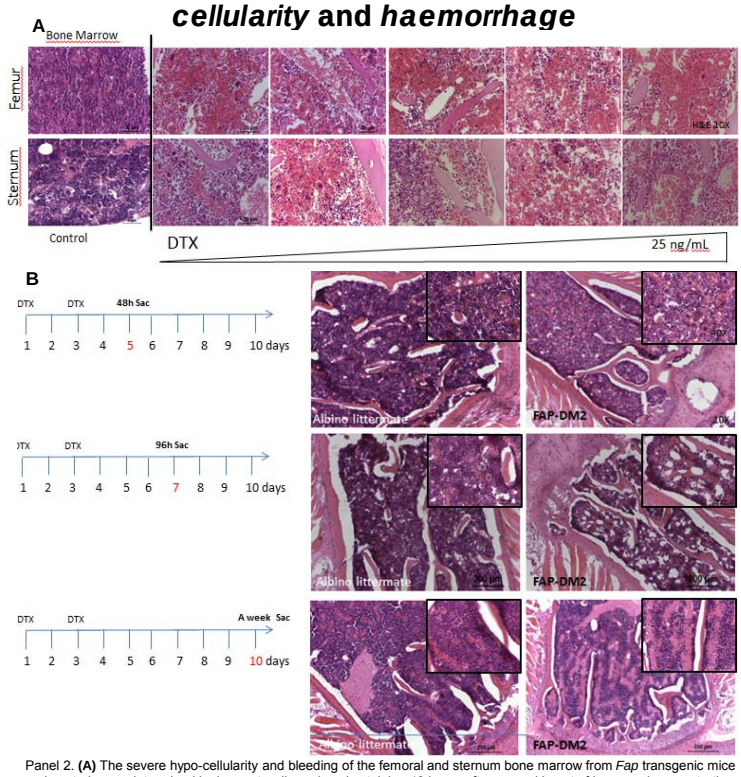
Methods and Results: FAP is ablated in stromal cell by using a simple and sensitive method to conditional cell ablation in transgenic mice called Toxin receptor-mediated conditional cell knockout (TRECK). FAP gene is modified genetically introducing the Diphtheria toxin Receptor (DTX-R) by using bacterial artificial chromosome (BAC). Ablation of FAP+ stromal cells in primary and secondary lymphoid organs (marrow and spleen) leads to reduced expression of CD362 and bleeding in the marrow. CD362 was identified as a stromal cell protein that labels MSC isolated from different species. Characterization, functionality and efficacy studies have shown the efficacy of CD362+ MSC in tissue repair, immunology suppression and homeostasis. At the moment we show that after FAP + cell ablation mouse MSC injection alleviate the bleeding in bone marrow replacing it by a extracellular matrix component.

Toxin receptor-mediated conditional cell knockout (TRECK).



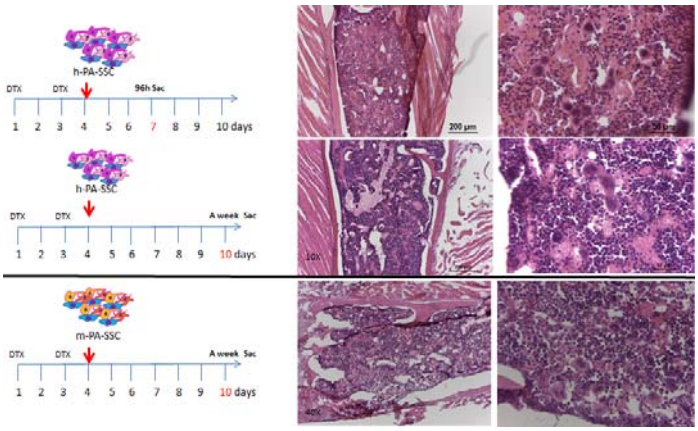
Panel 1. **(A)** Schematic draw taken from *Saito M. Nature 2001* showing the Toxin receptor-mediated conditional cell knockout (TRECT) procedure. **(B)** Schematic draw taken from *Roberts EW. J Exp Med. 2013* to show the transgene construction for *Fap*.

FAP+ stromal cell ablation cause severe *hypo-cellularity* and *haemorrhage*



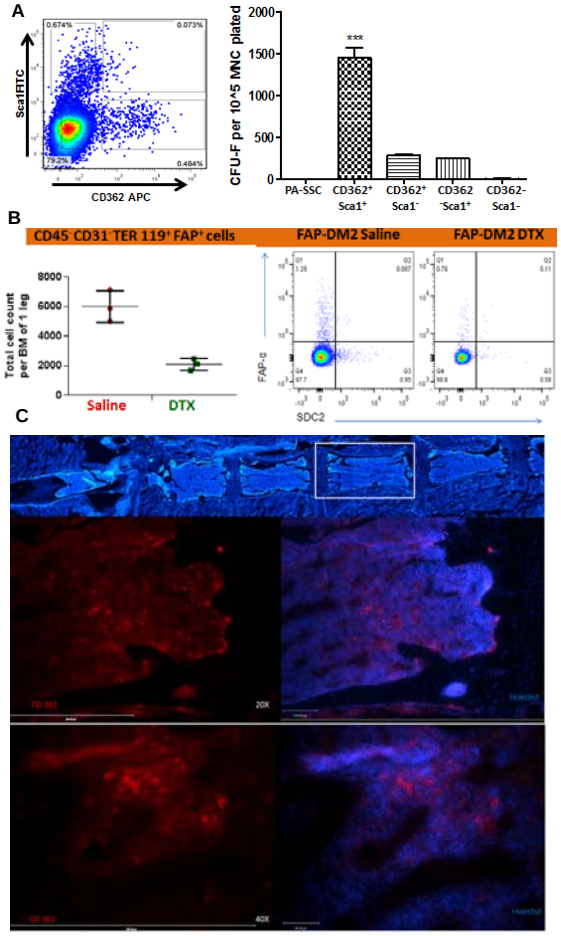
Panel 2. **(A)** The severe hypo-cellularity and bleeding of the femoral and sternum bone marrow from *Fap* transgenic mice and controls was determined by haematoxylin and eosin staining 48 hours after second boost of increased concentrations of DTX. (From 5 to 25 ng/mL). **(B)** Extra cellular matrix production was evidenced by haematoxylin and eosin staining 48, 96 hours and a week scarified after two DTX injections (25ng/mL).

Could be the phenotype rescued by Stromal stem cell administration?



Panel 3.. A549 cells stably transduced with plasmid expressing NFκB-luciferase and stimulated with 10ng/ml of TNFa & IL1β for 24hrs. **(A)** A549-NFκβ -luc were tranduced with Adenovirus expressing CD362 display reduced NFkB activation. **(B)** A549 cells were incubated with increasing amont of recombinsnt extracelluar CD362 (SDC2) for 24hrs displayed reduced activation of NFkβ in response to TNF-α/IL1-β activation.

CD362 protein decreases when the FAP+ stromal cells are ablated



Panel4.. In C57BL/6 mouse marrow, CD362+CD45- MNC express Sca1 **(A)** and enrich for CFU-F **(B)** (***p≤0.001). CD362+Sca1+ MSC reduce syngeneic CD4+ T lymphocyte proliferation in response to αCD3/APC stimulation **(C).** CD362 is expressed throughout murine marrow **(D).**
Figure 2. In C57BL/6 mouse marrow, CD362+CD45- MNC express Sca1 **(A)** and enrich for CFU-F **(B)** (***p≤0.001). CD362+Sca1+ MSC reduce syngeneic CD4+ T lymphocyte proliferation in response to αCD3/APC stimulation **(C).** CD362 is expressed throughout murine marrow **(D).**

Conclusions:

1. FAP+ stromal cell ablation model cause severe damage in the bone marrow.
2. The hypo-cellularity and haemorrhage phenotype in bone marrow can be changed by administrating mouse plastic adherent SSC.
3. CD362 a new stromal cell marker decrease after FAP + stromal cell ablation.

DECIDE
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