Oncostatin M is a novel niche factor that restrains haematopoietic stem cell mobilisation in response to G-CSF and CXCR4 antagonist Plerixafor

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Introduction

G-CSF and Plerixafor are currently being used in clinic for haematopoietic stem cells (HSPC) into the blood for transplantation. We investigated the role of pro-inflammatory cytokine Oncostatin M (OSM) on HSPC mobilisation.

Research objectives

1) Effect of OSM receptor (OSMR) gene deletion and OSM trap on HSPC mobilisation
2) Effect of OSM effect on HSC function (cell cycle, homing and chemotaxis)

Methods

Recombinant human G-CSF (Filgrastim, Amgen) was injected twice daily subcutaneously at 125 µg/kg; control mice received an equivalent volume of saline. AMD3100 octohydrochloride (Tocris Bioscience) was injected intraperitoneally as a single 16 mg/kg dose corresponding to 10 mg/kg of AMD3100 base. Tissues were harvested 1 hr after Plerixafor injection. Recombinant mouse OSM trap or control trap was injected at 1.5 mg/kg retro-orbitally starting the day before G-CSF administration and then once daily every day during G-CSF course (125µg/kg G-CSF twice daily for 3 days).Bone marrow, spleen or blood were processed for colony forming cells (CFC), ELISA, flow cytometry, immunohistochemistry, qRT-PCR and RNA sequencing.

Results

Figure1: G-CSF increases OSM protein in humans and mice.

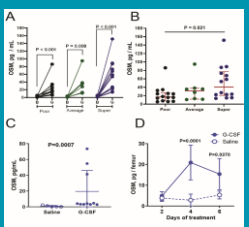

Fig1:A) OSM protein concentration in plasma of healthy human donors prior to and following G-CSF administration. B. Association between levels of CD34⁺ HSPC mobilisation and OSM concentration in healthy human donors. OSM protein concentration in blood (C) and BM extracellular fluids (D) of C57BL/6 mice mobilised with G-CSF for 4 days.

Figure2: Deletion of OSM receptor (*Osmr*$^{-/-}$) increases HSPC mobilisation in response to G-CSF.

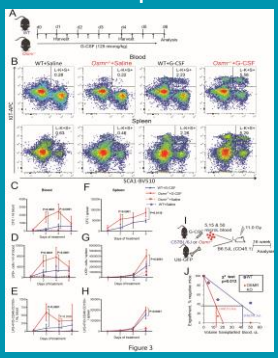

Fig2:A) C57BL/6 (WT) or Osmr-/- mice were treated with G-CSF or saline for indicated durations. B) Representative flow cytometry dot plots showing the enhanced HSPC mobilisation of LKS+HSPCs. C-H) Numbers of CFC, LKS+ HSPC and HSCs in blood and spleen in Osmr-/- and WT mice.

Figure3: In vivo neutralisation of OSM with OSM trap enhances HSPC mobilisation in response to G-CSF

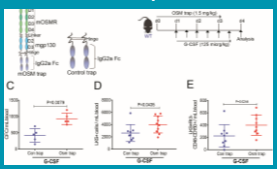

Fig3:A) Schematic of recombinant mouse control and OSM-trap. B) Schematic of OSM-trap and G-CSF administration. C-E) Numbers of CFC, LKS+ HSPC and HSCs in blood.

Figure4: Deletion of Osmr gene enhances HSPC mobilisation in response to Plerixafor.

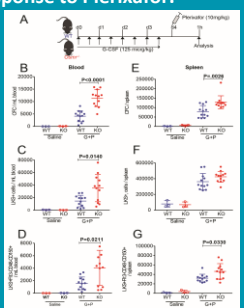

Fig4:A) Schematic of G-CSF and Plerixafor administration. B-G) Numbers of CFC, LKS+ HSPC and HSCs in blood and spleen *in Osmr*$^{-/-}$ and WT mice.

Figure5: HSPCs from Osmr-/- display enhanced chemotaxis in vitro and reduced BM homing in vivo.

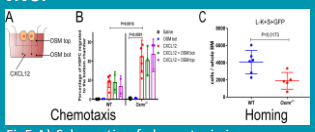

Fig5:A) Schematic of chemotaxis in response to CXCL12 gradient. Kit+-enriched BM cells from WT or Osmr-/- mice were seeded in top chamber and CXCL12 was seeded in bottom chamber. B) Percentage of LKS+HSPCs migrated into the bottom chamber from each genotype. C) Quantification of donor GFP+LKS+HSPCs in BM of non-irradiated G-CSF treated WT and *Osmr*$^{-/-}$ recipients 20 h post transplantation.

Figure6: Differential expression of genes in sorted LKS+Flt3-CD48 HSCs from BM of naïve WT and Osmr-/- mice.

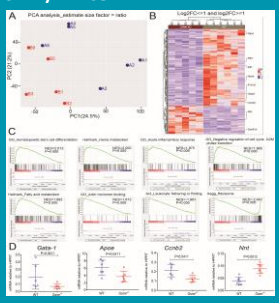

Fig6:A) Principal component analysis (PCA) plot of all samples analysed by RNA sequencing. Each genotype is colour coded, blue is WT and red is *Osmr*$^{-/-}$. B) Heat map and hierarchical clustering of differentially expressed genes (Log2(fold change)≥1.8 with FDPp<0.05). C) GSEA plots with highest and lowest normalised enrichment scores using GO, Hallmark and KEGG gene sets. D) validation of differentially expressed genes by RT-qPCR on LKS+Flt3-CD48-HSCs sorted from BM of naïve WT and *Osmr*$^{-/-}$ mice.

Conclusion

1) OSM provides a negative feedback acting as a brake on HSPC mobilisation in response to G-CSF and Plerixafor.
2) OSM attenuates HSC chemotactic response to CXCL12 and increases homing to the BM.
3) RNA sequencing suggests that HSCs from *Osmr*$^{-/-}$ mice have altered cytoskeleton reorganisation, energy usage and cycling.

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