Notch signalling and stem cell ageing: insights from the ReSinAge project

Haematopoietic stem cells are responsible for the production of all the different cell types that constitute the blood, and their maintenance and differentiation must be tightly regulated during the whole life of an organism. Exciting new data emphasise that central aspects of blood ageing, ranging from inflammaging and immunosenescence to clonal haematopoiesis, are mechanistically linked to dysfunction and ageing of other tissues, supporting a central role for the haematopoietic system in this context (Andersson, Mejia-Ramirez and Florian, 2025). Recent findings within ReSinAge highlight a novel and unexpected involvement of reduced Jag2 Notch ligand expression in the HSC niche, which induces myeloid-biased HSC expansion and clonality upon ageing (Matteini *et al.*, 2025).

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Importantly, we have demonstrated that the alteration of the bone marrow (BM) microenvironment-driven Notch signalling activation determines the acquisition of intrinsic phenotypes of ageing in HSCs, opening up new targets to rejuvenate HSCs (Matteini *et al.*, 2025).

Ageing of the haematopoietic stem cell niche

Ageing of the haematopoietic system is characterised by phenotypic and functional impairments that are driven by alterations of haematopoietic stem cells (HSCs) and of the bone marrow niche. HSCs are responsible for the production of all the different cell types that constitute the blood, and their maintenance and differentiation must be tightly regulated during the whole life of an organism (Andersson, Mejia-Ramirez and Florian, 2025). Mounting evidence supports that the haematopoietic system might be driving healthspan and lifespan of the whole organism.

So far, several strategies have been proposed to rejuvenate stem cells (Matteini, Montserrat-Vazquez and Florian, 2024); however, their rejuvenating effects remain under debate. Our team showed that it is possible to rejuvenate HSCs by inhibiting the activity of the small RhoGTPase Cdc42 by using CASIN (Cdc42 Activity Specific Inhibitor). Cdc42 regulates cell polarity, and it is more active in aged murine and human HSCs compared to young stem cells. Cdc42 inhibition in aged HSCs by CASIN treatment restores cellular and epigenetic polarity of aged blood stem cells to levels similar to those present in young HSCs, and the ability of aged HSCs to regenerate the bone marrow upon transplantation (Florian *et al.*, 2012; Amoah *et al.*, 2022). Importantly, we demonstrated that transplanting rejuvenated HSCs into immunocompromised aged mice is sufficient to increase the health and lifespan of the entire organism (Montserrat-Vazquez *et al.*, 2022).

Interestingly, during this study, an important HSC phenotype stood out: the localisation in the BM of HSCs in aged mice treated with CASIN. HSC localisation in the BM is not random and reflects their regenerative capacity (Saçma *et al.*, 2019). Upon ageing, HSCs lose their proximity to arteries and the bone surface (endosteum) (Maryanovich *et al.*, 2018; Saçma *et al.*, 2019), while aged CASIN-rejuvenated HSCs were found closer to arterioles and to the endosteum, the same niches occupied by young HSCs, indicating that CASIN restores a youthful localisation (Montserrat-Vazquez *et al.*, 2022). Upon ageing, the expression of the Notch ligand Jagged2 (Jag2) decreases at BM arteries and NesGFP+ cells, while it is retained in the sinusoidal compartment. Interestingly, the HSCs that have divided less over time and possess the higher regenerative capacity in aged mice are those in proximity to Jag2+ sinusoids. Blocking sinusoidal Jag2 signalling by injecting in vivo Jag2 blocking antibodies displaces HSC from sinusoids and increases their proliferation (Saçma *et al.*, 2019).

Notch signalling: trans-activation and cis-inhibition

Notch signalling is one of the most highly conserved signalling pathways along phylogeny and plays a key role during the developmental process and in adult cell differentiation (Bigas and Espinosa, 2018). Notch signalling is activated in neighbouring cells and has an extremely complex regulatory system that allows the space and time-precise activation of this signalling. Notch is mainly activated in the cell that expresses one of the 4 Notch receptors (NotchR1-4), defined as the signalling-receiving cell, by the physical interaction of the receptor with one of the 5 Notch ligands (Dll1, 3, 4; Jag1-2) expressed by the signalling sending cell. The physical interaction between the ligand and the receptor leads to the cleave and release of the cytosolic domain of the receptor called Notch Intracellular Domain (NICD), which translocates to the nucleus and activates the genes under its control. This activation system is named trans-activation, as the source of Notch activation comes from a different cell than the one that will activate Notch. In specific conditions, the signalling-receiving cell can express both Notch receptors and Notch ligands simultaneously. In this case, the ligand can directly interact with the receptor expressed on the same cell, leading to Notch signalling inactivation, a phenomenon known as cis-inhibition (Sjöqvist and Andersson, 2019; Thambyrajah and Bigas, 2022).

The ReSinAge study: aged stem cells and Notch switching

The **ReSinAge project**, funded by the European Research Council (ERC), aims to continue studying the effects of ageing in HSCs to disclose the molecular mechanisms responsible for blood stem cell ageing, thereby opening up new rejuvenation strategies. Our manuscript 'A Notch trans-activation to cis-inhibition switch underlies haematopoietic stem cell ageing' (Matteini *et al.*, 2025) investigated the role of Notch signalling in HSCs ageing. Notch signalling plays a pivotal role in HSC development (Thambyrajah and Bigas, 2022). During the early phases of the development of the haematopoietic system, Notch signalling is strongly activated to promote HSC emergence from endothelial precursors localised in the Aorta Gonad Mesonephros (AGM), but its activation is subsequently downregulated through cis-inhibition to promote the maintenance of HSC fate (Souilhol *et al.*, 2016; Thambyrajah *et al.*, 2024). Despite several pieces of evidence highlighting that Notch signalling activation in young adult HSCs regulates

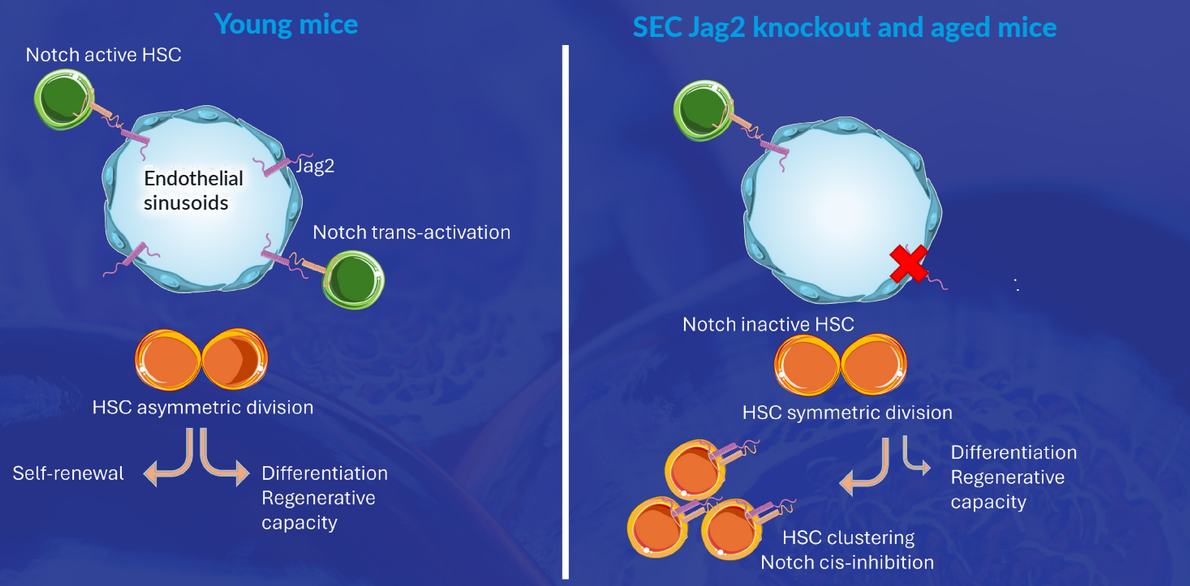

Figure 1: Comparison of Notch signalling in young versus aged and sinusoidal endothelial cell Jag2 knock-out (SEC-Jag2KO) haematopoietic stem cells (HSCs). Young HSCs near endothelial sinusoids activate Notch via Jagged2, supporting asymmetric division and regeneration. In aged or Jag2-deficient environments, Notch becomes inactive, leading to cis-inhibition, clustering and reduced regenerative capacity.

HSC regenerative capacity upon homeostasis and myeloproliferative stress (Guo *et al.*, 2017; Poulos *et al.*, 2017), the role of Notch signalling during HSC ageing is still far from being completely understood. Our paper filled this knowledge gap by showing that Notch signalling plays a key role during ageing and by revealing a switch from trans-activation to cis-inhibition, which promotes HSC ageing.

By taking advantage of a Notch reporter mouse model sensitive to fast Notch activation-inactivation cycles (Imayoshi *et al.*, 2013) and a highly sensitive 3D-quantitative histological approach (iFAST3D) to analyse cell localisation in the BM and cell-cell interactions (Saçma *et al.*, 2022), we first assessed Notch signalling activation in young HSCs. From this analysis, we observed that Notch signalling is not homogeneously activated and that two HSC populations exist: one with active Notch signalling (Notch active HSCs) and another with inactive Notch signalling (Notch inactive HSCs). Notch active HSCs display a specific localisation in proximity to arteries and sinusoids. As arteries and sinusoids are two of the main niches expressing Jag2, we wondered whether Jag2 expression from the endothelial cells might promote Notch activation in HSCs. To assess this hypothesis, we transplanted haematopoietic cells expressing a Notch-reporter into a mouse model where Jag2 is deleted only in sinusoidal endothelial cells (SEC Jag2 knock-out). After the induction of Jag2 deletion in sinusoidal endothelial cells, Jag2 is significantly downregulated selectively at sinusoids without major alterations of sinusoidal network morphology and structure. Interestingly, sinusoidal Jag2 deletion reduces Notch activation in HSCs, displaces them from the sinusoids, reduces Cdc42 polarity, increases the frequency of HSCs, and reduces their ability to regenerate the BM upon transplantation. These results indicate that Jag2 expressed from sinusoidal endothelial cells promotes Notch trans-activation in HSCs, regulates their perisinusoidal localisation, polarity, and sustains HSCs' long-term regenerative capacity.

Moreover, we observed that sinusoidal Jag2 deletion alters HSC divisional symmetry. Young HSCs mostly divide asymmetrically, with one daughter cell inheriting more H4K16ac than the other. Upon ageing, HSC divisional symmetry switches, favouring mainly symmetric division (in this case, both daughter cells inherit the same amount of H4K16ac) over asymmetric ones (Florian *et al.*, 2018). Interestingly, sinusoidal Jag2 deletion favours HSC symmetric divisions, recapitulating another phenotype of aged HSCs.

Thanks to an additional mouse model that selectively labels HSC and their progeny (Gazit *et al.*, 2014; Chapple *et al.*, 2018), we tested whether altering HSC divisional symmetry might result in an altered HSC fate through lineage tracing. Our findings support that sinusoidal Jag2 deletion alters HSCs, priming them upon division, which results in an expansion of myeloid-biased HSCs and a reduction in committed progenitors, indicating that Jag2 from sinusoidal endothelial cells regulates HSC fate and differentiation output long-term and collectively recapitulates several phenotypes of aged HSCs.

Importantly, upon ageing, the fraction of Notch-inactive HSCs increases, and HSCs are observed in close proximity to one another. This phenotype is typical of aged HSCs and is termed 'HSC clustering' (Florian *et al.*, 2018; Saçma *et al.*, 2019; Wu *et al.*, 2024). On the contrary, Notch active HSCs are always single HSCs and selectively retain sinusoidal proximity, confirming that sinusoidal Jag2 expression is critical to preserve perisinusoidal localisation of HSCs upon ageing. Interestingly, aged HSCs increase the expression of Jag2 themselves without modifying the expression of Notch receptors compared to young cells. Of note, Jag2 overexpression is recapitulated in young mice upon sinusoidal Jag2 deletion, indicating that the absence of Notch trans-activation from Jag2-expressing endothelial cells triggers Jag2 expression in HSCs. Jag2-expressing aged HSCs are also frequently clustered, suggesting that Jag2 expression may promote aged HSC clustering. To test this, we deleted Jag2 in HSCs, and we transplanted them into SEC Jag2 knock-out mice. Jag2 deletion in HSCs impeded HSC clustering induced by sinusoidal Jag2 deletion, showing that Jag2 expression in HSCs is essential to promote HSC clustering.

As in aged mice, both Notch-inactive HSCs and Jag2-positive HSCs are in clusters. We wondered whether Jag2 expression in *cis* (meaning on the same HSCs expressing Notch receptors) might trigger Notch inactivation by cis-inhibition. Therefore, we analysed Jag2 expression in aged HSCs of the Notch reporter mouse model using iFAST3D. We observed that Notch-inactive HSCs express Jag2 and are mainly clustered, confirming that Jag2 expression in aged HSCs cis-inhibits Notch signalling and that is essential for HSC clustering.

Towards targeted rejuvenation strategies

Collectively, we showed that Notch activity in HSCs is not homogeneous and depends on BM localisation, and that Notch-inactive HSCs expand in aged mice. The reduction of BM Jag2 expression at sinusoids decreases Notch activity, sinusoidal localisation of HSCs, cell polarity, symmetry of division, fate commitment and regenerative capacity. Notch inactive HSCs, which expand upon ageing, upregulate Jag2 expression, which induces stem cell expansion and clustering associated with Notch inactivation. Collectively, we demonstrate a key role of Notch signalling for adult HSCs. We show that the reduction of sinusoidal Jag2 expression underlies a switch from Notch trans-activation to cis-inhibition in aged HSCs. Moreover, our data show that a Notch signalling-mediated crosstalk between extrinsic BM signals and HSC intrinsic features contributes to the maintenance of HSC regenerative capacity throughout life, paving the way to a novel targeted approach to counteract HSC ageing.

References click here

PROJECT SUMMARY

Elderly cancer patients suffer high haematopoietic toxicity upon chemotherapy. ReSinAge aims to boost haematopoietic recovery and increase the survival after chemotherapy in the elderly by improving the regenerative capacity of the aged haematopoietic stem cell niche. In detail, we aim to disclose the functional interplay between blood stem cells and their niche, and its targeting as an innovative strategy to overcome chemotherapy toxicity and increase the survival of elderly cancer patients.

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PROJECT LEAD PROFILE

Dr Florian started her independent career focusing on stem cell ageing as Emmy Noether (DFG) junior group leader at the Institute of Molecular Medicine, University of Ulm (Ulm, Germany) in 2016. At the end of 2018, she was appointed by the Programme for the Clinical Translation of Regenerative Medicine in Catalonia (P-CMRC) and the Programme of Regenerative Medicine at IDIBELL (Barcelona, Spain). In 2023, she became an ICREA Research Professor (ICREA). Dr Florian's lab focuses on understanding cellular and molecular mechanisms of somatic stem cell ageing, supporting the development of new therapeutic strategies to preserve the regenerative capacity of stem cells over time and to limit or prevent the development of age-related disorders and extend healthspan and lifespan.

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