

Chosen for an Oral Presentation

Absence or blockage of E-selectin-mediated cell adhesion delays hematopoietic stem cell (HSC) turn-over and enhances chemoresistance.

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The behaviour of a hematopoietic stem cell (HSC) is regulated by its immediate micro-environment or niche. We have identified a novel function for the adhesion molecule E-selectin which is constitutively expressed on bone marrow (BM) vasculature. Using mice knocked-out for E- ($E^{-/-}$) or P-selectin ($P^{-/-}$), we investigated whether selectin absence alters HSC behaviour in vivo.

We found HSC cycling in the absence of E-selectin to be significantly delayed 2.5-fold in BrdU incorporation assays compared to either $P^{-/-}$ or WT (mice were administered BrdU for 3 days then BrdU incorporation in BM Lineage⁻KIT⁺Sca1⁺(LKS⁺)CD34⁻ or LKS⁺CD48⁻CD150⁺ cells measured). To confirm these findings, LKS⁺ cells were stained with rhodamine123, a vital dye retained by metabolically active cells but not quiescent HSC. More LKS⁺ cells from $E^{-/-}$ mice were rhodamine dull ($34\pm 2\%$) than WT ($23\pm 1\%$; $p=0.037$) confirming that a greater proportion of HSC from $E^{-/-}$ mice are quiescent.

We then determined whether administration of E-selectin antagonists alone could similarly delayed HSC turnover. Mice were administered the glycomimetic GMI-1070, for set periods of time before harvest. We found HSC turnover to be significantly delayed following GMI-1070 administration (1.4 fold less BrdU incorporation, $p=0.011$) with a concomitant 1.4-fold increase in the number of Rho123 dull LSK+ quiescent HSC per femur ($p=0.020$).

Non-cycling, quiescent HSC are known to be more resistant to chemotherapy and irradiation. Indeed 7 days following 5-FU administration (150mg/kg), we found that $E^{-/-}$ mice had faster BM HSC recovery /less HSC damage compared to WT mice, both by phenotype analysis and in a competitive long-term reconstituting assay. Following 5-FU administration the number of reconstituting units/femur in WT mice decreased 5.1-fold but only decreased 2.3-fold in similarly treated $E^{-/-}$ mice.

Interestingly, when mice were pre-treated with the glycomimetic E-selectin antagonist GMI-1070, before 5-FU, there was significantly enhanced blood neutrophil recovery compared to mice administered 5-FU alone (blood neutrophils were $710\pm 205 \times 10^3/\text{mL}$ with GMI-1070, compared to $234\pm 141 \times 10^3/\text{mL}$ without, at day 9 post-5-FU, $p=0.0001$). Similarly when mice were severely irradiated (9Gy) and test bleeds performed weekly, a more rapid haematopoietic recovery was observed in $E^{-/-}$ compared to WT mice. In summary, we have identified a novel function for the adhesion molecule E-selectin. HSC turnover is dramatically reduced in $E^{-/-}$ mice an effect that can be replicated by transient administration of E-selectin antagonist mimetics. Furthermore blood leukocyte and HSC numbers recover faster following cytotoxic or irradiation injury in the absence or blockage of E-selectin-mediated cell adhesion. Thus E-selectin may well be a crucial component of the proliferative HSC niche regulating HSC turnover and blockage of this adhesive interaction may represent a promising treatment for the protection of HSC's during chemotherapy. GMI-1070 has completed Phase I clinical trials and its activity described here suggests a possible clinical use for this indication.