Improved Engraftment of Bone Marrow and Mobilized Peripheral Blood Stem Cells in a Fanconi Anemia Murine Model.

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Fanconi anemia (FA) is a genetic syndrome characterized by development of progressive bone marrow failure and cancer predisposition. The difficulty in harvesting FA HSC and their fragility during subsequent *in vitro* manipulations has proven a confounding factor in attempted gene therapy of this disease. Mobilization of HSC/P in FA patients is poor (Croop *et al*. Blood, 2001) probably due to HSC deficiency. We have previously shown that the genetic deletion of the Rac GTPases 1 and 2 results in an increase in circulating hematopoietic stem cells and progenitors (HSC/P) (Gu *et al*. Science 2003). In addition, administration of a single dose of a small molecule inhibitor of Rac GTPases, NSC23766, results in a transient mobilization of engraftable stem cells (Cancelas *et al*. Nat. Med., 2005). Here, we analyzed the role of NSC23766 in mobilizing HSC/P in FA A (*Fanca-/-*) mice (Cheng *et al*., Hum. Mol. Genet., 2000; kindly provided by M. Grompe, OHSU). First, we validated that this murine model of FA demonstrated a stem cell phenotype by a competitive repopulation assay of BM HSC. We found that *Fanca-/-* HSC contribute decreased chimerism in short-term engraftment (52.6 2.6% donor engraftment) compared to wild-type (WT) controls (63.8 1.0%, respectively, $p < 0.005$). BM and spleen homing of *Fanca-/-* HSC/P at sixteen-hours post infusion was not impaired (7.0% in BM and 6.1% in spleen) compared to WT mice (7.8% in BM and 5.4% in spleen) and there was no difference in expression of CXCR4, a_4-integrin, a_5-integrin and L-selectin between Lin^-/c-kit^+/Sca-1^+ BMC and mobilized PBC derived from *Fanca-/-* and WT mice, also supporting an intrinsic HSC defect. We then analyzed the ability of NSC23766, alone or in combination with G-CSF, to mobilize HSC. We observed that *Fanca-/-* mice also show an impaired mobilization response to G-CSF administration (200 mcg/Kg/day for five days), which can be partially rescued by administering a single dose of NSC23766, 6 hours before peripheral blood harvest (Table1). We additionally demonstrated the impaired engraftment of *in vitro* manipulated *Fanca-/-* BMC in a competitive transplant assay. This engraftment defect could be completely ameliorated by treatment with Diprotin A (5.9±2.0% donor engraftment untreated vs. 12.0± 4.4% treated; p value = 0.01). Diprotin A is an inhibitor of CD26 peptidase which has been shown to cleave SDF1alpha/CXCL12. The combined use of G-CSF and NSC23766 may constitute a future novel approach to induce mobilization of Fanconi anemia HSC and, when coupled with Diprotin A treatment, could act to enhance the engraftment of cells undergoing genetic correction.

Table 1. Competitive Repopulating Units (normalized to WT GCSF)

	Short Term Engraftment (+1 month)	Long Term Engraftment (+4 months)
WT GCSF (%)	100 ±34.08	100 ±52.34
WT GCSF + NSC 23766 (%)	77.43 ±37.11	53.34 ±54.61
Fanca-/- GCSF (%)	11.25 ±5.92 *	2.54 ±2.59 **
Fanca-/- GCSF + NSC 23766 (%)	24.45 ±1.89	39.01 ±29.47 ***

* p value < 0.05, compared to WT GCSF; ** p value < 0.001, compared to WT GCSF, *** p value < 0.05 compared to Fanca-/- GCSF