

Molecular Targeting Strategy to Enhance Fanconi Anemia Type C-deficient Hematopoietic Stem Cell Function

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Objective: Significant evidence supports a role for enhanced oxidant and TNF-alpha mediated loss of hematopoietic stem/progenitor cells in the pathogenesis of bone marrow (BM) failure in Fanconi anemia (FA). Previously, we demonstrated that both H₂O₂ and TNF-alpha treatment of *Fancc* -/- murine embryonic fibroblasts results in increased activation of the stress activated kinases, p38 and c-Jun N-terminal kinase (JNK). In addition, we showed that oxidant and TNF-alpha induced apoptosis of *Fancc* -/- hematopoietic progenitors is reduced to WT levels by inhibiting p38 or JNK activity. Given these previous findings, the aims of the current study were to determine whether p38 and/or JNK inhibition enhance *Fancc* -/- hematopoietic stem cell (HSC) function.

Methods: The methods employed to address these aims took advantage of a model system developed by our group showing that culturing *Fancc* -/- BM cells results in loss of HSC reconstituting ability. We examined whether culturing *Fancc* -/- cells with the p38 inhibitor SB203580 would enhance repopulating ability in competitive repopulation assays. Thus, two test cell populations (*Fancc* -/- cells cultured with vehicle control and *Fancc* -/- cells cultured with SB203580) were mixed with BoyJ competitors before transplanting into 5-7 irradiated recipients/group. Two independent primary transplants and one secondary transplant have been conducted. Using a similar strategy one competitive repopulation experiment has been performed using a JNK inhibitor.

Results: The addition of SB203580 to cultured *Fancc* -/- cells significantly increased repopulating ability compared to *Fancc* -/- cells cultured with vehicle control. The improvement in reconstitution of *Fancc* -/- cells was sustained over 12 months in primary recipients (p<0.05). Multilineage analysis revealed enhanced lymphoid and myeloid reconstitution supporting an increase in HSC function with inhibition of p38 in cultured cells. Twelve months post-transplantation all mice exhibited normal peripheral blood counts, BM and spleen histology. Similar results were obtained in both primary transplants conducted. Secondary transplants are currently underway, but preliminary data suggest sustained donor chimerism in secondary recipients as well. The primary transplant to test whether inhibiting JNK in cultured *Fancc* -/- cells enhances reconstituting ability is ongoing. However, early data do not detect differences in the repopulating ability of *Fancc* -/- cells cultured with a JNK inhibitor or vehicle control.

Conclusions: Taken together these data suggest that enhanced p38 activity may account for the loss of *Fancc* -/- reconstituting cells in culture and emphasize the importance of conducting *in vivo* studies to assess the ability of small molecule inhibitors to enhance HSC function.

Translational Applicability: Identification of molecular mechanisms involved in BM failure has the potential to lead to translational therapies targeting pathways aberrantly activated in FA cells. Novel information from these studies may lead to the development of therapeutic approaches to prevent/treat BM failure/myeloid malignancies in FA.