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Loss of Tcf7 diminishes hematopoietic stem/progenitor cell function

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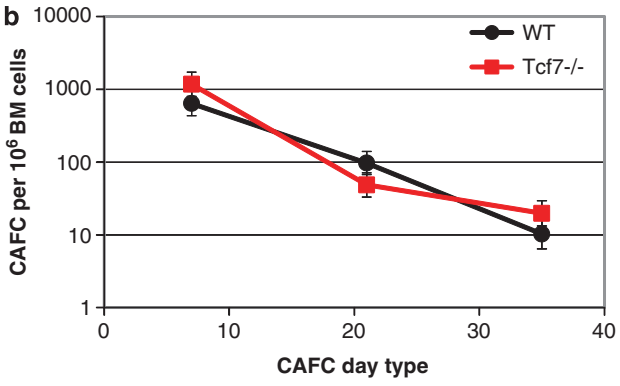
The canonical Wnt-β-catenin pathway is an evolutionarily conserved and tightly regulated pathway in development. Activation of this pathway occurs upon binding of a soluble Wnt protein to a membrane-associated receptor, and leads to the disruption and inhibition of a protein complex responsible for the phosphorylation and breakdown of β-catenin. Inhibition of this so-called destruction complex, composed of the tumor suppressor Apc, the Ser-Thr kinases Gsk-3β and CK-I, and the scaffold and tumor suppressor protein Axin, results in stabilization and (nuclear) accumulation of β-catenin. Stabilized β-catenin forms a bipartite transcription factor with the Tcf-Lef family of transcription factors (including Tcf7, Tcf7l1, Tcf7l2 and Lef1) to activate a Wnt-controlled gene expression program.[1]

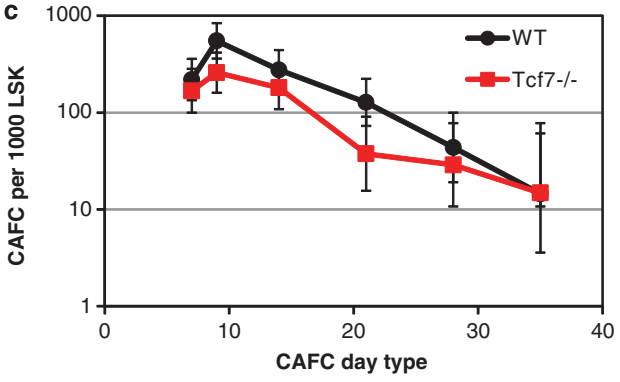
In the hematopoietic system, a role for Wnt signaling was first demonstrated during T-cell development in the thymus.[2] Subsequently, opposing effects of Wnt signaling on hematopoiesis have been reported. For example, Mx-Cre-mediated deletion of β- or β- and γ-catenin did not effect hematopoiesis, but stabilized forms of β-catenin resulted in either enhancement of hematopoietic stem cell (HSC) function and maintenance of an immature phenotype or exhaustion of the HSC pool.[3–8] These differences might be explained by different levels of Wnt pathway activation. By using various targeted hypomorphic Apc alleles and a conditional deletion allele of Apc, which generates different Wnt signaling levels, it has been shown that different, lineage-specific Wnt dosages regulate HSCs, myeloid precursors and T lymphoid precursors during hematopoiesis.[9]

The *Tcf7* gene is a complex gene of which several different isoforms, including isoforms that lack the β-catenin binding domain, have been found. *Tcf7* has been reported to be a β-catenin-Tcf7l2 target gene and to act as a feedback repressor of β-catenin-Tcf7l2 target genes. In this way, Tcf7 may assist Apc to suppress malignant transformation of epithelial cells.[10] Tcf7 expression is most abundantly expressed in T-lymphocytes, but is also expressed in HSCs.[11,12] B6-Tcf7-deficient mice develop a progressive block in the early thymocyte development, but are fully immunocompetent and live for over 1 year.[2,13] Here, we report the impact of loss of Tcf7 on HSC and progenitor cells *in vitro* and *in vivo*.

a

	BMC/HL	%LSK
WT	$5.0x10^7$	0.24
$Tcf7^{-/-}$	$4.9x10^7$	0.25





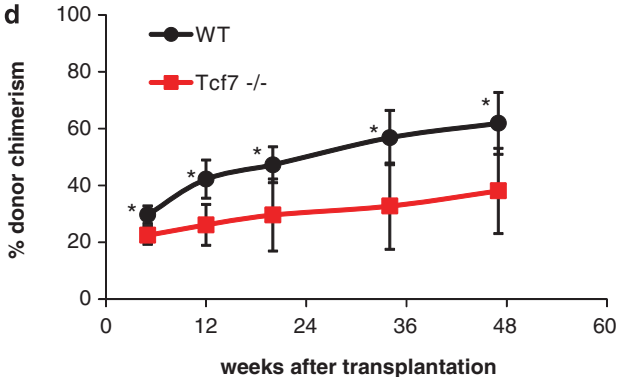


Figure 1. Comparison of LSK frequency and functionality *in vitro* an *in vivo* between WT and B6Tcf7−/− mice. (**a**) BM cellularity and LSK frequency (pooled from three mice). BM cells (**b**) and purified LSK (**c**) were plated in CAFC cultures, and CAFC frequency was determined at different culture times. (**d**) *In vivo* functionality of unseparated BM cells (2 million) transplanted in competition with normal BM cells (1 million). The percentage of donor chimerism was determined in blood on nucleated blood cells by flow cytometry and the % donor chimerism was calculated (% donor/(% donor + % competitor)).

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Tcf7-deficient mice, which have been backcrossed at least 10 times onto the C57BL/6 background, were found to have normal peripheral blood counts (not shown). To determine the number of cells with a $Lin^{-}Sca\text{-}1^{+}\text{-}ckit^{+}$ (LSK) phenotype, containing all long-term repopulating cells, bone marrow (BM) cells were immunophenotyped and LSK cells were evaluated *in vitro* assays. The number of BM cells per hindlimb was comparable between B6-wt and B6-Tcf7−/− mice. In addition, the number of cells with the primitive LSK phenotype per hindlimb was also comparable between wild-type and Tcf7−/− mice (Figure 1a).

Next, we determined CAFC (Cobblestone Area-Forming Cell Assays) frequencies in BM isolated from B6-wt and B6-Tcf7−/− mice. The CAFC assays were used to quantify the number of hematopoietic progenitors cells or stem cells in the fluorescence-activated cell sorting -purified cell populations at specific time points. To this end, test cells were seeded in a limiting dilution manner in 96-well plates containing a preestablished FBMD-1 stromal layer, as described.[14] The number of CAFC per 10^6 BM cells at 7, 21 and 35 days was not different between wild-type and B6-Tcf7−/− mice (Figure 1b). CAFC frequencies at later time points (days 28/35) are a surrogate measure of HSC/progenitor cell function. Also, the frequency of all CAFC subsets per 1000 LSK cells was not statistically significant different between B6-wt and B6-Tcf7−/− mice (Figure 1c).

To determine the impact of loss of Tcf7 on *in vivo* stem cell repopulation ability, we performed competitive transplantation assays. After conditioning with 9.5 Gray total body irradiation, 1×10^6 BM cells from B6-Tcf7-deficient or B6-wt mice were transplanted with 2×10^6 BM cells of B6.SJL (CD45.1) competitors. Interestingly, chimerism levels of mice transplanted with B6-Tcf7−/− BM cells was significantly lower compared with mice transplanted with B6-wt mice (Figure 1d). This indicates that loss of Tcf7 significantly diminishes the repopulation ability, suggesting impaired stem cell potency after loss of Tcf7. In addition, the competitive repopulation index (CRI) was calculated. The CRI was calculated by taking the ratio of white blood cells derived from B6-Tcf7−/− or B6-wt BM cells to competitor BM cells in the circulation and dividing it by the ratio of B6-Tcf7−/− or B6-wt BM cells to competitor BM cells transplanted. The CRI was approximately four times higher in B6-wt BM cells than in B6-Tcf7−/− BM cells, suggesting approximately fourfold reduction in repopulating ability in B6-Tcf7−/− BM cells. In summary, our findings demonstrate that B6-Tcf7−/− mice have normal *in vitro* but diminished *in vivo* functionality of the hematopoietic stem/progenitor compartment. Multiple explanations, including homing, engraftment, niche utilization, cycling rate might be responsible for this phenotype. In addition, as the LSK population had no reduced activity *in vitro*, it seems most likely that the quality of LSK was affected, rather than a decreased number of cells with a more primitive phenotype within the LSK population.

Various publications have reported evidence that Wnt signaling is involved in self-renewal of mouse HSCs. Indeed, the level of Wnt signaling was found to be important for the effects on hematopoiesis.[9] However, limited data are available on the downstream effectors of the canonical Wnt signal-transduction pathway, such as the Tcf/Lef1 transcription factors, on hematopoiesis. Interestingly, using the EMD multipotent hematopoietic precursor cell line as a model, it has been shown that Tcf7 is the most downregulated transcription factor when CD34+ cells switch into CD34− cells.[12] Additionally, Tcf7 knockdown experiments and gene set enrichment analysis suggested that Tcf7 has a dual role in promoting the expression of genes characteristic of self-renewing CD34+ cells while repressing genes activated in partially differentiated CD34− state.[12] These *in vitro* data support our data that loss of Tcf7 diminishes HSC/progenitor cell function, but the exact mechanism remains to be determined.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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