BIO REGULATES THE *EX VIVO* EXPANSION AND FUNCTION OF HEMATOPOIETIC STEM CELLS BY INHIBITING GSK-3β

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Hematopoietic stem cells (HSCs) have been applied in clinic settings for treating hematologic diseases, including leukemic disorders, immune deficiencies, and hemoglobinopathies. Umbilical cord blood(UCB) is an important source of HSCs. However, the low frequency of HSCs per unit of UCB remains a big hurdle to their wider applications. Wnt/β-catenin pathway plays important roles in the self-renewal of HSCs *in vivo*, but the roles of Wnt/β-catenin signaling on *ex vivo* expansion of HSCs remains controversial. GSK3β is the major regulator of Wnt pathway. Here, we evaluate the effects of 6-bromoindirubin-3'-oxime (BIO), a GSK3β inhibitor, on *ex vivo* expansion characteristics and regenerative potential of (UCB)-derived $CD34^+$ cells.

First, the expansion of $CD34^+$ cells at the presence of different concentrations of BIO was examined. Compared to the control, BIO treatment favored the expansion of $CD34^+$ cells within the 4-day culture, while there was a trend toward repress the expansion of $CD34^+$ cells on day 7. These results indicated that Wnt/β-catenin pathway is potentially related to the expansion of $CD34^+$ cells. Subsequently, western bolt analysis showed that the amounts of β-catenin, phosphorylated GSK3β, and downstream effector Cyclin D1 were unchanged by BIO treatment at days 4 of culture. Notably, the β-catenin accumulated gradually with the prolongation of culture time peaking at day 7 with BIO treated. However, transcription factor TCF showed significant decrease at days 7 compared to days 4 of culture. Remarkable increase in total CFU number was observed in BIO-treated cells relative to control, mainly because the frequency of CFU-GM (granulocyte, macrophage) was significantly higher. The expansion fold of total cells derived from BIO-cultured CD34+ cells favored the secondary expansion of total cells at day 4 and 14.

In conclusion, Wnt/β-catenin signaling agonist BIO regulated the expansion of $CD34^+$ cells and differential expression of key factors in Wnt signaling pathway in a β-catenin-dependent fashion during *ex vivo* culture. Furthermore, BIO-expanded $CD34^+$ cells showed better multilineage commit potential and secondary expansion ability, which provides a valuable guidance for optimizing *ex vivo* culture.

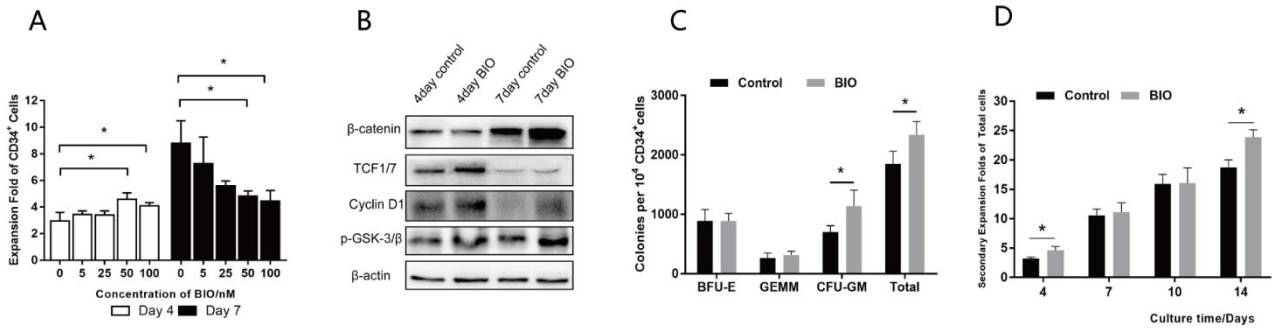

*Figure 1 (A)The effect of BIO on expansion fold of $CD34^+$ cells (*p<0.05 , n=3). (B) Western blot analysis of for β-catenin, TCF, Cyclin D1 and phosphorylated GSK3β in CB $CD34^+$ cells. (C, D) CFU and the secondary expansion ability analysis of expanded-$CD34^+$ cells (*p<0.05 , n = 3 experiments).*