The Role of BMP4 in the *Ex vivo* Expansion of Cord Blood Haemopoietic Stem Cells

Jonathon F. Hutton B.Sc. (Hons)

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Abstract

Establishment of conditions supporting haemopoietic stem cell (HSC) maintenance and expansion *ex vivo* is critical for wider clinical application of cord blood (CB) transplantation. AFT024 is a murine fetal liver cell line that expands primitive haemopoietic cells via a process that is not understood. Here we show that bone morphogenetic protein (BMP) 4, which is a member of the transforming growth factor-beta (TGFβ) super-family of pleiotropic regulators, is produced by AFT024 and contributes significantly to the maintenance of co-cultured CB-derived primitive cells. Significant amounts of BMP4 mRNA are produced by the supportive AFT024 stromal cell line and secreted BMP4 protein accumulates in AFT024 conditioned medium. Blockade of BMP4 activity in this co-culture model using neutralising BMP4 monoclonal antibody reduced expansion of primitive CB cells based on phenotypic ($CD34^+CD38^-$) and functional criteria (LTC-IC), and significantly reduced the capacity of the cultured CB stem cells to support repopulation in the NOD-SCID xenograft model. BMP4 is therefore an important growth factor for maintenance of HSC that contributes to the unique properties of the AFT024 stroma non-contact culture. Addition of supplemental BMP4 to clinical *ex vivo* cultures also increased maintenance of primitive haemopoietic cells in serum- and stroma-free defined culture conditions. Thus, BMP4 can contribute to HSC maintenance both in an established long-term co-culture model and in a clinical *ex vivo* expansion setting. On the basis of these studies BMP4 should be considered as a component in future CB *ex vivo* expansion protocols for use with other haemopoietic cytokines.

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