Emerging Technologies and Novel Research Avenues

Feasibility of Flow Cytometric Analysis of nPOD Bone Marrow Specimens

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Purpose: Diabetes-induced dysfunction of bone marrow (BM)-derived stem cells (SCs)/progenitor cells (PCs) may contribute to peripheral cardiovascular complications and microvascular disease. We recently reported the occurrence of microangiopathy in BM of diabetic mice, which causes critical reduction in perfusion, oxidative stress, PC loss through apoptosis and disturbance of endothelial cells (EC)-PCs interaction. We now aim to verify if similar adverse remodelling affects the vascular and haematopoietic components of BM in diabetic patients. Therefore, we tested the feasibility of flow cytometry analysis (FACS) of antigenically-defined cellular subpopulations in human BM samples from nPOD project.

Methods: The effect of diabetes on BM-PCs was assessed analyzing three nPOD iliac crest BM cases: 1) case 6126, not diabetic (ND) male (M), age 25 years, 2) case 6161, Type 1 diabetic (T1D), M, age 19 years, and 3) case 6132, T2D, female, age 52 years. Frozen specimens were thawed following nPOD instructions. FACS analysis was performed to detect distinct cellular subpopulations in the 7AAD-negative fraction (please see Table 1 below for details on the antigenic profile of subpopulations tested).

Summary of Results: Although freezing/thawing procedure resulted in depletion of the total number of cells available for the analysis, the percentage of dead cells was relatively low. FACS analysis confirmed that thawed cells were alive (7AAD negative) in all the three samples tested. We were also able to detect all the cellular subpopulations under investigation and obtained percentages in line with current literature data (please see Table 2 below for FACS results).

Conclusions: We successfully verified a standard operating protocol to thaw nPOD BM specimens in perspective of further cytometric analysis. Samples were adequate to measure the percentages of different populations of BM cells. These preliminary feasibility studies open up the possibility of exploiting human samples from nPOD to investigate the action of diabetes in inducing alterations in BM composition. Moreover, in consideration of the high cell viability, the current method may be exploited to explore BM PC function including the angiogenic properties of early and late EPCs.

(Tables on next page)

Table 1. Definition	Antigenic profile
Hematopoietic PCs	$CD34^{pos}$, $CD133^{pos}$, and c-kit ^{pos}
T-lymphocytes	$CD45^{pos}/CD3^{pos}$
B-lymphocytes	$CD45^{pos}/CD19^{pos}$
Natural Killer cells (NKs)	$CD3^{neg}/CD56^{pos}/CD16^{pos}$
Mesenchymal Cells (MSCs)	$CD73^{pos}/CD105^{pos}/CD90^{pos}/CD34^{neg}/CD45^{neg}$
Endothelial cells (ECs)	$CD45^{neg}/CD31^{pos}/CD144^{pos}$
Early endothelial PCs (eEPCs)	$CD34^{pos}/CD14^{pos}/CD45^{pos}/KDR^{pos}/CXCR4^{pos}$
Late EPCs (lEPCs)	$CD34^{pos}/CD14^{neg}/CD45^{neg}/KDR^{pos}/CXCR4^{pos}$

Table 2. FACS (% of indicated MNC population)	ND	T1D	T2D
$CD34^{pos}$	2.8	0.6	0.9
$CD133^{pos}$	0.2	0.3	0.3
c-kit ^{pos}	2.3	1.6	0.9
T-lymphocytes	17.3	11.2	23.8
B-lymphocytes	8.5	3.2	4.6
NKs	3.3	3.7	8.2
MSCs	0.003	0.03	0.01
ECs	0.03	0.43	0.5
eEPCs	0.04	0.13	0.2
lEPCs	0.02	0.13	0.1