STEM CELL BIOLOGY

321 HUMAN UMBILICAL CORD BLOOD (HUCB) DERIVED STEM CELLS AND THEIR POTENTIAL IN TREATING INHERITED SKIN DISEASES AND PROMOTION OF WOUND HEALING

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Recessive Dystrophic Epidermolysis bullosa (RDEB) is a severe inherited skin blistering disease caused by mutations in the Col7A1 gene. COL7A1 is synthesized by keratinocytes and fibroblasts and is a major component of anchoring fibrils that extend from basement membrane to papillary dermis. A recent animal study suggested that the hematopoietic enriched cells ameliorated the blistering phenotype in the RDEB mice. However, only limited donor epithelial engraftment and COL7A1 protein were observed and the possibility of rare stem cells co-purifying with enriched cells cannot be excluded. As multiple populations of primitive stem cells with multi-lineage differentiation potential have been identified from HUCB, including unrestricted somatic stem cells (USSCs), they may offer potential for the treatment of RDEB.

Goal: To isolate USSCs from HUCB and determine the potential in treating RDEB and promoting wound healing.

Methods: USSCs were initiated from HUCB in 30% FBS and 10^-7M dexamethasone. Q-RT-PCR was utilized to assess gene expression of Nanog, Oct4, Sox2 and Col7A1. DNA methylation at the enhancer and promoter of both Oct4 and Nanog genes was analyzed by bisulfate sequencing. The expression of CCR2 was analyzed by immunocytochemistry.

Results: HUCB-USSCs are lineage negative and share overlapping but distinct surface markers with MSCs. They express a low but consistent level of Nanog, Oct4 and Sox2. Significantly, the Nanog and Oct4 expression in USSCs is about 20-400-fold higher than that in human fibroblasts. Their level was further increased 10-fold following the treatment with DNA methylation inhibitor, 5-azaCydine. An average of 65% and 47% of the CpGs were unmethylated in the enhancer and promoter of the Nanog gene respectively, while 56% and 80% were unmethylated at those of the Oct4 gene. We also showed that USSCs express Col7A1, at a level comparable to human keratinocytes, suggesting that USSCs, once engraft in the skin, could rescue the defective anchoring fiber formation by secreting COL7A1.

In addition, we demonstrated that USSCs express the CCR2, a receptor for several chemoattractant proteins, such as MCP-1, that have been implicated in regulating hematopoietic stem cells (HSCs), but neither pathway is sufficient for self-renewal. Using a HSC and progenitor (HSPC)-specific conditional mutant mouse model, we studied Pten deletion combined with β-catenin activation. In contrast to single mutants, double mutant mice exhibit a novel phenotype including expansion of phenotypic HSCs without extensive differentiation. Together, the PTEN/Akt and Wnt/β-catenin signaling pathways interact to drive HSC expansion by inducing proliferation while simultaneously inhibiting apoptosis and blocking differentiation—demonstrating the necessity of complementary cooperation between the two pathways in promoting self-renewal. Mechanistically, this is accomplished by upregulation of the HSC self-renewal related gene Hoxb4 and Inhibitor of differentiation 2 (Id2) combined with downregulation of the apoptosis inducer st100/8A49 in double mutants. Informed by this genetic model, we developed an ex vivo HSC expansion protocol. In serial transplantation experiments, simultaneous activation of both PTEN/Akt and Wnt/β-catenin pathways achieves long-term engraftment equivalent to a one-hundred fold greater dosage of uncultured HSCs—demonstrating unprecedented expansion of long-term repopulating HSCs. This expansion is achieved using serum-free media with low doses of cytokines but without using feeder-cell layers or permanent genetic manipulation, making our protocol ideal for potential translation into the clinic.

322 ASSESSMENT OF THE BONE MARROW COMPARTMENT VOLUME AND EFFECT OF CHEMORADIOThERAPY USING 18F-FLUORothYMIDINE

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Evaluation of the bone marrow compartment currently consists of methods such and fluorodeoxyglucose (FDG) PET imaging and MRI, cumverting issues of donor availability particularly in light of recent volcanic ash). During 2006 to 2009, our cell processing laboratory cryopreserved 30 allogeneic and 35 autologous PBSC. It was noted that the post-thaw viable CD34+ recovery was lower in allogeneic PBSC products (median 63%, range 16-92) than autologous (72%, 11-91; p < 0.0004). Here this study was aimed to determine factors that influence post-thaw CD34+ recovery.

We analysed data from all cryopreserved allogeneic and autologous PBSC, with the aim of determining the effect of cryopreserved nucleated cell concentration (NCC), neutrophil content, and time from collection to cryopreservation.

Univariate analysis demonstrated weak inverse correlations were between viable CD34+ recovery and NCC (Spearman ρ = -0.20, p < 0.0001), collection to freeze time interval (ρ = -0.10, p = 0.048), laryngeal carcinoma (n = 10). Radiation therapy(RT) to cervical spine was consistent with clinical observation with a decline in marrow SUV from 3.0 ±1.34 before RT to 1.94 ± 0.60 (P(0.013) after RT. Chemotherapy resulted in no significant change in SUV when tested 6 months after last dose of chemotherapy with a mean SUVmax of 4.99 ± 1.15 pre-chemotherapy and mean SUVmax of 5.28 ± 1.50 post-chemotherapy (P(0.21)). We suggest that FLT-PET imaging may be a useful tool for the assessment of the marrow compartment and may be useful for assessment of marrow injury following radiotherapy.

323 WNT/β-CATENIN SIGNALING COOPERATES WITH PTEN/P38/AKT SIGNALING IN VIVO AND EX VIVO TO PROMOTE HEMATOPOIETIC STEM CELL SELF-RENEWAL AND EXPANSION

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Although self-renewal is the central property of stem cells, the underlying mechanism remains inadequately defined. Separately, the PTEN/P38/Akt and Wnt/β-catenin signaling pathways have been implicated in regulating hematopoietic stem cells (HSCs), but neither pathway is sufficient for self-renewal. Using a HSC and progenitor (HSPC)-specific conditional mutant mouse model, we investigated whether β-catenin activation. In contrast to single mutants, double mutant mice exhibit a novel phenotype including expansion of phenotypic HSCs without extensive differentiation. Together, the PTEN/Akt and Wnt/β-catenin pathways interact to drive HSC expansion by inducing proliferation while simultaneously inhibiting apoptosis and blocking differentiation—demonstrating the necessity of complementary cooperation between the two pathways in promoting self-renewal. Mechanistically, this is accomplished by upregulation of the HSC self-renewal related gene Hoxb4 and Inhibitor of differentiation 2 (Id2) combined with downregulation of the apoptosis inducer st100/8A49 in double mutants. Informed by this genetic model, we developed an ex vivo HSC expansion protocol. In serial transplantation experiments, simultaneous activation of both PTEN/Akt and Wnt/β-catenin pathways achieves long-term engraftment equivalent to a one-hundred fold greater dosage of uncultured HSCs—demonstrating unprecedented expansion of long-term repopulating HSCs. This expansion is achieved using serum-free media with low doses of cytokines but without using feeder-cell layers or permanent genetic manipulation, making our protocol ideal for potential translation into the clinic.

324 LOWER VIABLE CD34 RECOVERY IN CRYOPRESERVED ALLOGENEIC PBSC COMPARED TO AUTOLOGOUS PBSC

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Allogeneic peripheral blood stem cells (PBSC) are cryopreserved less often than autologous harvests. The use of cryopreserved allogeneic HPC is becoming increasingly common for storage of excess HPC or T cells for donor lymphocyte infusions, in addition to circumstances issues of donor availability particularly in light of recent worldwide events (Sept 11, swine flu and flight disruptions due to volcanic ash). During 2006 to 2009, our cell processing laboratory cryopreserved 30 allogeneic and 35 autologous PBSC. It was noted that the post-thaw viable CD34+ recovery was lower in allogeneic PBSC products (median 63%, range 16-92) than autologous (72%, 11-91; p < 0.0004). Here this study was aimed to determine factors that influence post-thaw CD34+ recovery.

We analysed data from all cryopreserved allogeneic and autologous PBSC, with the aim of determining the effect of cryopreserved nucleated cell concentration (NCC), neutrophil content, and time from collection to cryopreservation.