"Human Cord Blood derived Stem Cells Differentiated into Hormone-Expressing Islet Cell-like Aggregates to Produce Insulin as an Alternative to Pancreatic transplant for diabetic and pancreatic Cancer Patients"

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Celprogen's strategies to differentiate progenitor stem cells derived from human cord blood into Islets of Langerhan Beta cells *in vitro* have been considered as an alternative to increase beta cell availability prior to subsequent transplantation for Diabetic and pancreatic cancer patients who have their Beta cell population compromised in some way. It has recently been suggested that nestin-positive cells could be multipotential stem cells capable of expressing endocrine markers upon specific stimulation. Here, we characterized short- term 30 days and long-term 60 days differentiation of stem cell cultures into Islets of Langerhan Beta cells that were pre-selected and expanded into nestin positive and CD34 positive stem cells derived from human cord blood.

Method:
The stem cells were collected from five different human subjects cord blood after normal term delivery, and the stem cells were processed from the cord blood with respect to pre-selected expression of nestin positive and CD34 positive stem cells. The differentiated stem cells into Islet of Langerhan Beta cells were characterized for their specific pancreatic cell types with confocal microscopy, bright field microscopy, and semi-quantitative RT-PCR.

Results:
The number of nestin-positive stem cells was found to be strikingly high in long-term expansion cultures. In addition, there was a large proportion (90.5%) of these nestin-positive stem cells and CD34 positive stem cells, present in long-term expansion culture. The proportion of insulin-positive cells was found to be high in short-term (up to 55days) cultures and declined thereafter, when cells were maintained in the presence of 10% serum in Celprogen's pancreatic stem cell growth medium, and concomitantly with the decrease in insulin and PDX-1 gene expression declined as well. Insulin and nestin positive cells co-expression was observed in these islet cells cultured for 45 days in culture. Upon long-term 60 days, sub-culturing of nestin-positive stem cells in Celprogen's stem cell growth medium, we observed reappearance of insulin expression at the mRNA level; when these cultures were shifted to Celprogen's serum free stem cell growth media for a month, then the expression of insulin, glucagon and somatostatin hormones were detected, indicating that manipulating the culture conditions with Celprogen's stem cell growth medium can modulate nestin-positive stem cell's fate to differentiate into pancreatic hormone specific cell types. The differentiation of nestin-positive stem cells that were plated onto Celprogen's pancreatic extra-cellular gel matrix revealed that these cells tend to aggregate to form islet-like clusters, similar to the CD34

positive stem cells but this is not sufficient to increase insulin expression upon short-term 30 days in culture.

Results:

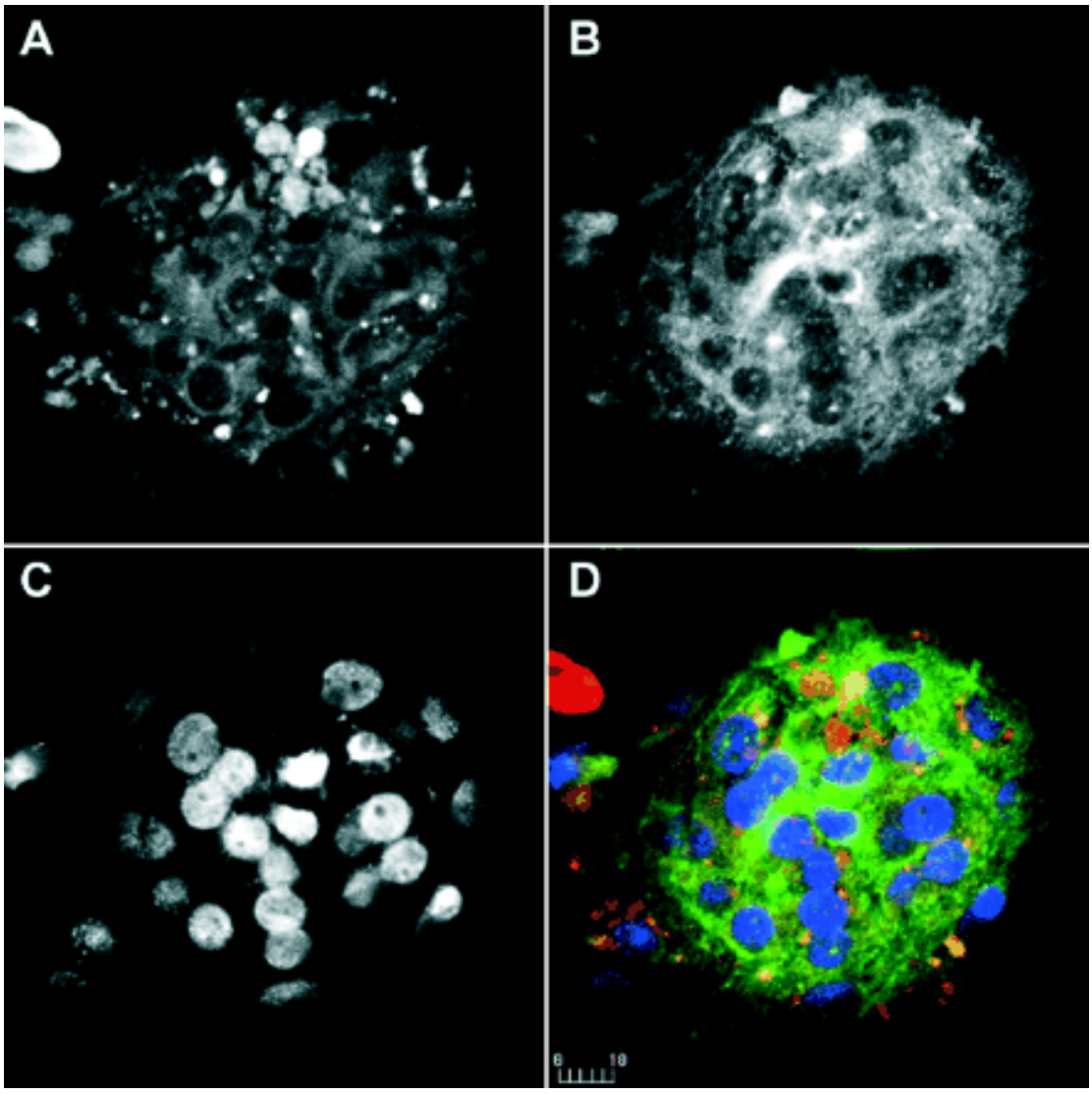

Figure 1 Confocal microscopy of triple-labeled immunofluorescence of cord blood derived stem cells differentiated into islets of Langerhan Beta cells cultured for 7days depicted for (A) insulin (green), (B) nestin (red) and (C) DAPI labeling for nuclei (blue). The superposition of A, B and C is shown in D. Insulin in the center of the islet (A), nestin-positive cells among the insulin-positive cells and of regions of co-localization of both insulin and nestin (in yellow). Scale bar in µm.

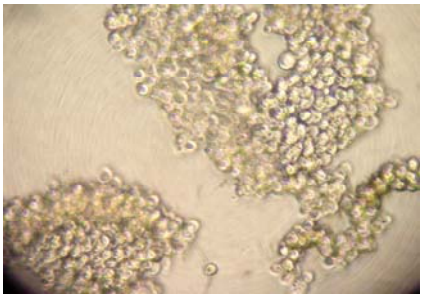

Figure 2: Human cord blood derived stem cells in culture day 14 stained with Crimson Belle stain, red stained cells are positive for insulin positive cells in cell cluster Bright field microscopy. The Crimson Belle stain was utilized initially to select viable stem cells that were positive for nestin and CD34 cells. The Crimson Belle stain was utilized to stain the stem cell clusters that were further stained with immunological probes for insulin islet of Langerhan Beta cells.

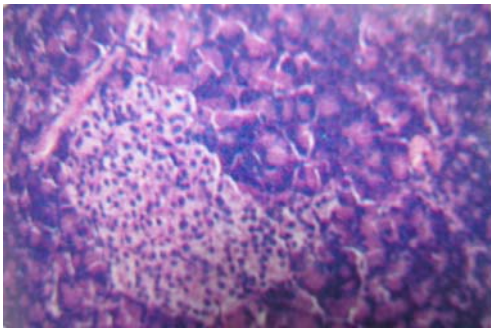

Figure 3: Islet of Langerhan cells derived from Human Cord Blood Stem Cells 30 days in culture stained with Hematoxylin and Eosin. The lightly stained area indicates the islet of langerhan cells.

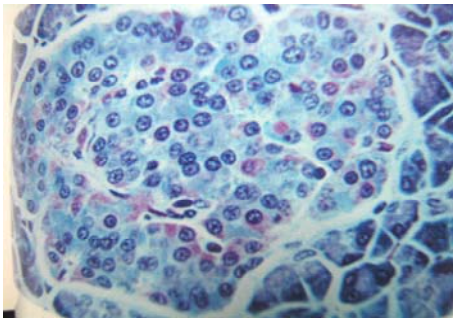

Figure 4: Blue stained cells positive for Somatostatin and red stained cells are positive for Insulin for differentiated Human Cord Blood derived stem cells at day 45 in culture maintained in Celprogen's pancreatic stem cell growth medium and cultured on Celprogen's pancreatic cell matrix.

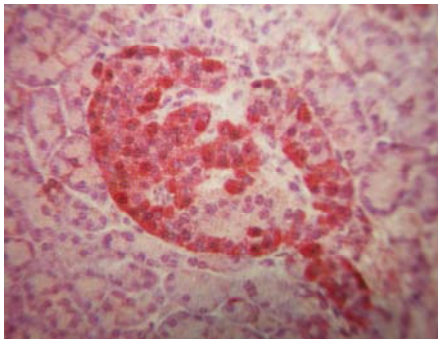

Figure 5: Glucagon positive cells take up the red immunospecific stain for glucagon alpha cells, stained on day 45 in culture of the trans-differentiated stem cells maintained in Celprogen's pancreatic stem cell growth medium and cellular matrix.

Table 1: Ability of Stem Cell trans-differentiated into Islet of Langerhan Beta cells to produce insulin when cultured for 60 days long-term culture with CELPROGEN pancreatic growth medium. Note the insulin production for up to 30 days in culture when initially maintained in CELPROGEN's pancreatic growth medium with serum (30 days in culture) and then transferred to CELPROGEN's pancreatic serum free growth medium for an additional 30 days without serum. Data represent the average±S.E.M.

Insulin secretion µ U / cell	Days in Culture		
	7	15	30
	11.7 ± 0.03	10.5 ± 0.04	9.2 ± 0.05
	11.2 ± 0.05	10.2 ± 0.03	9.0 ± 0.03
	11.9 ± 0.02	10.7± 0.03	9.5 ± 0.03
	11.6 ± 0.04	10.3 ± 0.05	9.1 ± 0.05
	11.3 ± 0.02	10.5 ± 0.02	9.0 ± 0.04

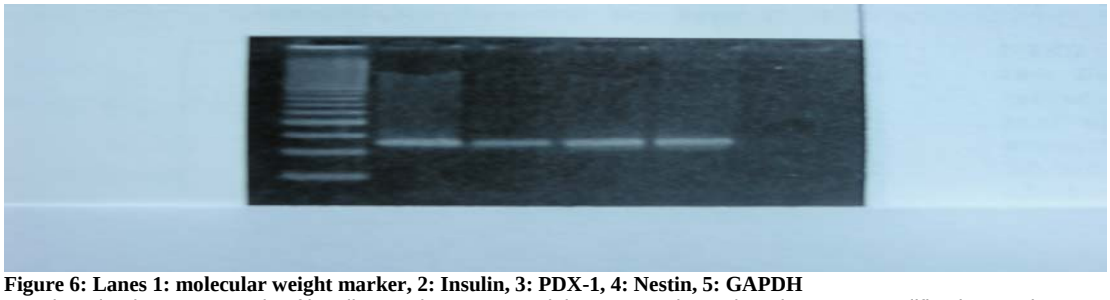

Figure 6: Lanes 1: molecular weight marker, 2: Insulin, 3: PDX-1, 4: Nestin, 5: GAPDH
Fractionation in agarose gels of insulin, nestin, PDX-1 and the GAPDH housekeeping gene amplification products at the exponential range of RT-PCR. Long-term cultures of human pancreatic islets, obtained from five different donors, cultured for 60 days long term culture onto coated plastic, showing that nestin-positive cells are present for long term culture of 60 days, but insulin is detectable only in the first month of culture. Insulin and PDX-1 were detected for up to 38 to 56 days in culture, with very low levels of transcripts being present after this period.

Conclusion:

From this study, we have demonstrated the possibility of pre-selecting and differentiating human umbilical cord blood stem cells *in vitro* into Islet of Langerhan cell-like aggregates capable of producing insulin. This provides the possibility of ex-vivo differentiation of cord blood stem cells into insulin-producing cells and subsequent transplantation into insulin-dependent diabetic and pancreatic cancer patients.