Isolation, characterization and comparative gene analysis of Endothelial Progenitor Cells from fetal and perinatal tissue

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ABSTRACT

Endothelial progenitor cells (EPCs) have become a population of great interest in recent years. Their potential use in the therapeutic development of a variety of postnatal diseases, including cardiovascular and peripheral vascular disorders and cancer are currently being studied extensively. However, few groups have looked into EPCs derived from fetal blood. Hence, The aim of this study is first to prove the presence of EPCs in fetal blood and to second isolate, culture and characterize EPCs derived from both fetal blood and cord blood for Oligonucleotide microarray analysis. This was done to provide some basic and comparative analysis on gene expression of fetal versus cord blood EPCs. Our final gene list includes VEGFR-2, Jagged 1, Stabilin 1 and IL-18 that were upregulated in fetal EPCs and BTG-1 (B cell translocation gene 1) that was upregulated in cord blood EPCs.

INTRODUCTION

Angiogenesis, which is based on sprouting from pre-existing vessels, plays an important role in a variety of physiological and pathological diseases in adulthood. Vasculogenesis is the formation of primitive vascular networks from endothelial progenitor cells (EPCs) or angioblasts. Increasing evidence are now pointing to the possibilities that vasculogenesis does occur in postnatal life.

Despite these findings, EPCs have not yet been fully defined. There have been discoveries of various types of EPCs with different characteristics. A subtype of EPCs, namely, Endothelial outgrowth cells (EOCs) have been reported to be a type of EPC population that appears after 7 days in culture and bear high proliferative potential. They are also characteristically exhibit cobblestone morphology. EOCs have been found in human bone marrow and more recently umbilical cord blood.

In this study, we test the hypothesis that EPCs are also present in fetal blood and that due to their early developmental stage, fetal EPCs have greater expansion potential. To investigate this, we isolated fetal CD31⁺ mononuclear cells and compared them with cord blood EOCs. We also compared the gene expression profiles by an Oligonucleotide microarray analysis on the EOCs.

This study lists a number of genes expressed differentially in the fetal and cord blood EOCs. This gene list may help navigate the search for the potential biomarkers and therapeutic targets to modulate vasculogenesis in adult life.

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MATERIALS & METHODS

All tissue samples were collected with approval and consent from the NUS Institutional Review Board (NUS-IRB). For each source, we obtained two samples. The EOCs were cultured in EGM-2 media. The experiment was carried out according to Figure 1.

RESULTS AND DISCUSSION

Figure 2. Isolated EOCs from fetal and umbilical cord blood.
(A) Cord Blood EOCs with cobblestone morphology at 4X and 40X magnification.
(B) Fetal Blood EOCs with cobblestone morphology at 4X and 40X magnification.
Distinct cobblestone shaped colonies (Fig. 2) appeared from fetal MNCs following 9-26 days of culture, whilst colonies appeared from cord blood between 19-28 days.

The growth kinetics and proliferation capacity of fetal and cord blood EOCs were assessed as well as compared (Table 1 &2). An unpaired t test was conducted (results not shown) to compare the mean doubling times at passage two between the two sources. The p value was < 0.05 and thus significantly different. For passage three, there was no significant difference between the two sources as p > 0.05. Hence, there are two possibilities—one being there was sample-to-sample variance and second being that fetal blood does not have higher proliferative capacity.

In this experiment, we used CD31$^+$ fetal EOCs to characterize a functional endothelial progenitor cell population. This was because CD31 was specific for endothelial cells. The drawback for using CD31 however was that fetal blood contained very few CD31$^+$ at the time of selection. This could be due to a few reasons. One would be that CD31 was not yet expressed in fetal EOCs. It has been found that in human embryoid bodies (hEBs), CD31 was not expressed until day 3 of differentiation and peaked between days 11-15. This also explains the low CD31 expression in fetal blood when analysed with flow cytometry as well as the less distinct cell-cell junctions observed in staining (Fig 3. C-D). Conversely, CD31 expression is found in cord blood derived endothelial cell types, in line with the chronology of development.

In our CFU-assay and Matrigel, fetal blood EOCs distinctly forms very large colonies as compared to minute colonies in cord blood. Fetal EOCs are able to form much more developed capillary tubes. Hence, fetal EOCs have a higher vasculogenic potential and ability to form colonies (Figure 4 &5).

EOCs were first characterized for CD34, CD31 and CD133 expression. It should be noted that only one sample of cord blood EOCs was analysed by flow cytometry.

It was surprising to find that CD31 was very highly expressed in umbilical cord blood whereas very lowly in fetal (unselected) blood. In CD31$^+$ fetal EOCs, there was a high expression of CD31. Fetal and CD31$^+$ EOCs both expressed CD34 whereas cord blood did not. CD133 was expressed in all three types of cells—cord blood, fetal blood and CD31$^+$ EOCs (Table 3-5).
In immunophenotyping, fetal blood EOCs expressed CD31, CD144, HLA I, HLA II, vWF, eNOS and also incorporated ac-LDL. Cord blood, similarly, expressed everything apart from HLA I (Fig. 3).

In this study, we were only able to analyze the differential gene expression of fetal against cord blood EOCs based on n=1, due to contamination and degradation of one sample of total RNA from each group (Table 6).

Out of all the genes, 628 genes were found to be upregulated in cord blood EOCs, whereas 507 genes were found to be upregulated in fetal EOCs. The upregulated genes for fetal blood include those known to be involved with vascular (blood vessel) morphogenesis: VEGFR-2 (type III KDR), IL-18 (interferon gamma-inducing factor), jagged 1 (Notch ligand) and stabilin 1 (scavenger receptor). Those genes upregulated for cord blood EOCs were found to be mainly involved in endothelial cell differentiation: BTG-1 (B cell translocation gene).

One of the genes identified to be upregulated in fetal blood EOCs is VEGFR-2. VEGFR-2 is a kinase insert domain receptor (a type III receptor tyrosine kinase) that has been found to be the major receptor for VEGF-A. It was discovered that mouse embryos lacking VEGFR-2 expression die at around 9 days of development and show no development of blood vessels or hematopoietic cells.

Another gene that we found to be interesting was interleukin-18. IL-18 is an interferon gamma-inducing factor and its signaling was found to evoke effectors that are involved in endothelial vascular development.

Thirdly, we also found the gene Jagged 1 to be upregulated. It is involved in Notch signaling, which plays myriad roles during vascular development and physiology. These roles include regulation of blood vessel sprouting and branching during both normal development and tumor angiogenesis.

Fourthly, in fetal blood EOCs, stabilin 1 was also found to be significantly upregulated. Stabilin 1 is selectively expressed in tissue macrophages; non-continuous endothelial cells and in endothelial cells of continuous origin during certain phases of vascular morphogenesis or angiogenesis, e.g. wound healing.

In cord blood EOCs, we found an interesting gene, which is upregulated as compared to fetal EOCs. It is the BTG-1 (B cell translocation gene). This gene is a member of an anti-proliferative gene family that regulates cell growth and differentiation. From the gene expression profiles, it is obvious that fetal blood EOCs are in the early developmental stage as the genes regulated are involved in formation and organization of the vascular system. Cord blood EOCs are on the other hand, are involved in processes of endothelial differentiation. Further experiments in the future would include real-time quantitative RT-PCR analysis to validate and profile the expression patterns of the five genes discussed above.

REFERENCES

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