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Metadichol® and CD34 Expression in Umbilical Cord Cells

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Abstract

Umbilical cord blood has found use in the clinic for more than 40 years in hematopoietic stem cell transplantation therapies to treat patients with bone marrow diseases or to reconstitute the bone of those cancer patients who had to have theirs wiped out to cure their leukemia or lymphoma. A feature is the presence of CD34 antigen in of hematopoietic stem and progenitor cells. These cells can differentiate and are self-renewing, multipotent stem cells that give rise to all blood cells of the immune system and erythrocytes), and lymphoid (T cells, B cells, and NK cells) lineages. This study describes increased CD34 gene expression in Umbilical Cord (UC) cells upon treatment with Metadichol which is an inverse agonist of AHR (Aryl Hydrocarbon Receptor). UC cells were subjected to treatment at one picogram, 100 picograms, 1nanogram, 100 nanograms and 1microgram per ml of Metadichol for 72 hrs. Cells treated at 1ng have shown the highest increase in expression of CD34 compared to untreated Control. The cells treated with 1pg, 100 picogram/ml demonstrated the multiplicity of CD34 expression as indicated by peak shift compared to treatment with 1ng, 100 ng, and 1 µg

Keywords: CD 34; Cord blood; Stem cells; Multipotency; Umbilical cord blood; Metadichol, AHR; Aryl Hydrocarbon receptor; VDR; RORC; Inverse agonist; TNF alpha; ICAM1

Introduction

Molecules that modulate adult or embryonic stem cell fate can facilitate the use of stem cell therapies to a multitude of diseases [1]. Well-known characterized adult stem cells are hematopoietic stem cells (HSC) [2]. The expected clinical potential has not materialized due to a lack of defined culture conditions for their expansion [3]. Human umbilical cord blood (CB) is rich in hematopoietic stem cells (HSC) similar to that in bone marrow. CB transplants are increasing annually around the world [4]. The growth of blood banks that store CB and clinical data that support that point to HLA mismatched transplants with low risk of b versus-host disease (GVHD) [5].

The broadest utilization of CB is limited by the low number of HSCs per unit, and most CB units have insufficient stem cells for adults. A lot of work has gone into developing technologies for *ex vivo* expansion of HSCs to enable CB transplants. A low number of hematopoietic stem and progenitor cells in cord blood units limit their widespread use in human transplant protocols.

A feature of hematopoietic stem and progenitor cells is the presence of CD34 antigen cells that are self-renewing, multi-potent stem cells that lead to all blood cells of the immune system and erythrocytes) and lymphoid (T cells, B cells, and NK cells) lineages [6]. The highly specialized cells that arise from HSC are essential in defending the body against infection and disease. Today bone marrow transplantations use stem cells and also make use of their potential for regeneration of damaged tissues. Cord blood cells have a relative long telomere DNA in comparison to their analogs from peripheral blood or bone marrow [7]. Cells from cord blood are capable of hematopoiesis for a longer time. They generate more divisions and produce a more significant number of progeny (daughter cells).

The increase in the expression of CD34 antigen mainly precedes cell differentiation. Transplants of hematopoietic cells have assumed an essential role in the treatment of diseases. They hold much promise for the clinical application involving gene therapy, tolerance induction to facilitate allogeneic or xenogeneic organ transplants. Recent work show the potential of HSC is limitless to generate whole organ systems [8-10].

Boitano and colleagues recently reported a purine derivative termed SR1 that significantly expands human CB HSCs in culture [11]. The authors screened a chemical library of 100,000 heterocyclic compounds for their ability to grow the numbers of CD34 CB cells in culture. They showed that SR1 supported a fifty-fold increase in CD34 cells. SR1 is an antagonist of the aryl hydrocarbon nuclear receptor protein (AhR), which usually mediates xenobiotic responses but have also been implicated in regulating hematopoietic stem/progenitor cells. Knockdown of the AhR resulted in a sustained proliferation of CD34 cells in culture.

A recent clinical trial of *ex vivo* expanded umbilical cord blood CD34 cells used SR-1 and demonstrated better engraftment and improved early recovery of leukocytes, suggesting that *ex vivo* expansion of HSCs might potentially be achievable [12,13]. Similarly, co-cultures of cord blood CD34 cells with mesenchymal stromal cells led to the expression of CD34 cells by a factor of 30.1, improving the time required for neutrophil engraftment to 15 days, compared to 24 days in recipients who received un-manipulated cord blood CD34 cells [14]. Growing evidence indicates that targeting metabolism and cellular stress for HSC expansion might potentially lead to successful HSC expansion approaches for transplantation therapies in the future [15,16].

Metadichol® [17] is a lipid emulsion of long-chain alcohols, and we have recently shown that it is an inverse agonist of AHR [18] the only one in medical literature. Given Boitano's work that AHR antagonists expand CD34 cells in UBC (umbilical cord cells), our results show that Metadichol which is a safe food-based ingredient can increase expression of CD34 in Umbilical cord blood at picogram levels.

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Experimental

All work was done by Skanda Labs, Bangalore India.

Reagents

Antibody: CD34-FITC conjugates: BD Pharmingen

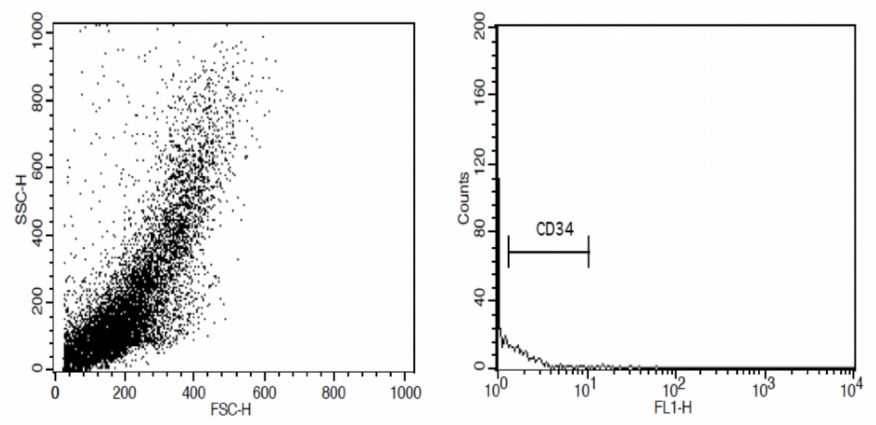
Cell Line: Umbilical Cord (UC) cells were sourced from Hi-Media labs. Germany, they were human Wharton's jelly cord blood cells. They were not frozen and expanded after five passages.

Procedure

Culture 1 × 106 cells in a 6-well plate containing 2 ml of complete media.After 24 h of incubation, cells are treated with 1 pg, 100 pg, 1 ng, 100 ng and 1µg in serum-free DMEM media and incubated for 72 h. After 72 Hr. of treatment, cells were collected and, pelleted cells at 4000 rpm for 5 minutes at room temperature and discard the supernatant. Washed the cell pellet twice with 1X PBS. The cell pellet suspended in 100 µL of Sheath fluid and incubated with CD34-FITC antibody for 20mins in the dark. Post incubation, the cells were once washed with 1X PBS and resuspended in Sheath fluid. The treated and untreated cell populations were determined using FACS Caliber (BD Biosciences, San Jose, CA).

Results

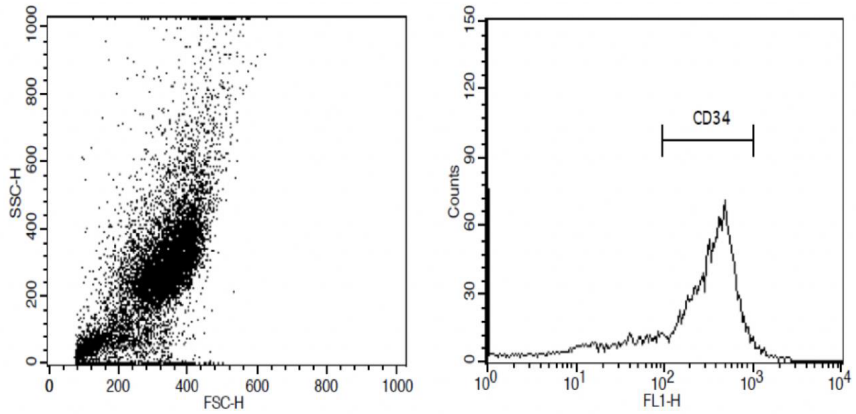
UC cells treated with Metadichol at 1 pg, 100 pg, 1 ng, 100 ng, and 1 µg of for 72 hrs. Cells treated at 1 ng has shown the highest increase in expression of CD34 (Figures 1-4, 82.14%) compared to



Histogram Statistics

Marker	Left, Right	Events	% Gated	% Total	Mean
All	1, 9910	10000	100.00	100.00	1.10
CD34	1, 11	503	5.03	5.03	2.17

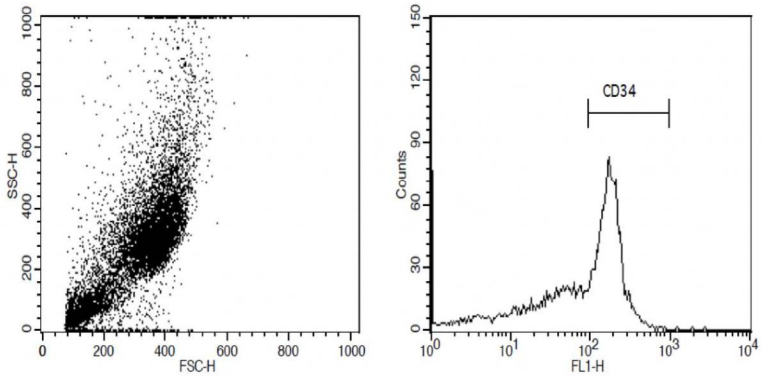
Figure 1: Control plots of UC cells.



Histogram Statistics

Marker	Left, Right	Events	% Gated	% Total	Mean
All	1, 9910	10000	100.00	100.00	313.21
CD34	96, 1018	7447	74.47	74.47	387.27

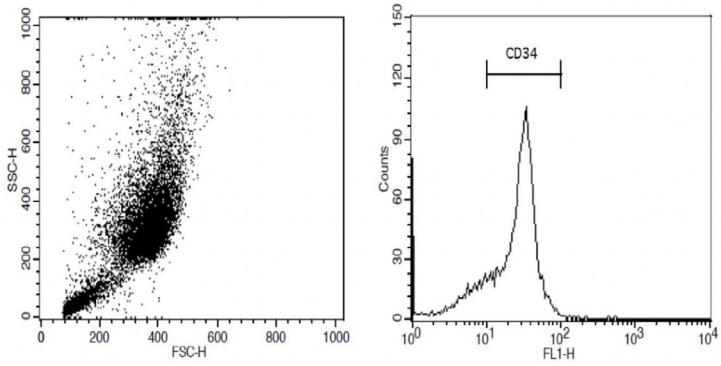
Figure 2: UC cells treated with 1 pg of Metadicol.



Histogram Statistics

Marker	Left, Right	Events	% Gated	% Total	Mean
All	1, 9910	10000	100.00	100.00	118.64
CD34	96, 973	5688	56.88	56.88	182.46

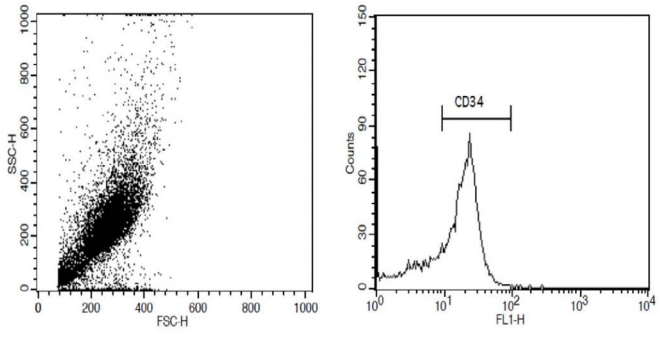
Figure 3: UC cells treated with 100 pg of Metadicol.



Histogram Statistics

Marker	Left, Right	Events	% Gated	% Total	Mean
All	1, 9910	10000	100.00	100.00	26.70
CD34	10, 100	8214	82.14	82.14	31.01

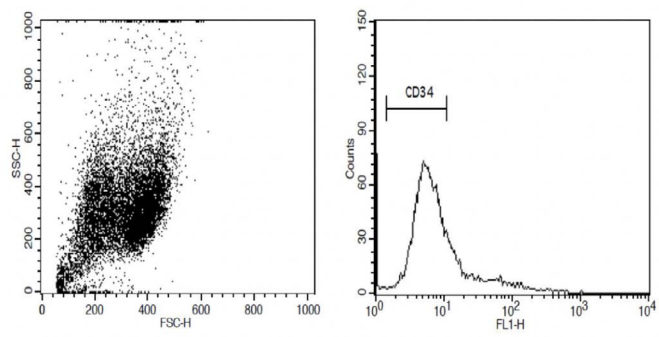
Figure 4: UC cells treated with 1 ng of Metadicol.



Histogram Statistics

Marker	Left, Right	Events	% Gated	% Total	Mean
All	1, 9910	10000	100.00	100.00	16.37
CD34	9, 96	6810	68.10	68.10	22.29

Figure 5: UC cells treated with 100 ng of Metadicol.



Histogram Statistics

Marker	Left, Right	Events	% Gated	% Total	Mean
All	1, 9910	10000	100.00	100.00	15.27
CD34	1, 11	7160	71.60	71.60	5.77

Figure 6: UC cells treated with 1 µg of Metadicol.

untreated Control. The cells treated at 1pg, 100pg has demonstrated the multiplicity of CD34 expression as indicated by peak shift (Figures 2 and 3) compared to treatment with 1 ng, 100 ng, and 1 µg (Figures 5 and 6). The fold increase is at various concentrations is difficult to calculate as the control cells, the signals for CD 34 look more like background noise. Nonetheless, the CD34 cell expression at 100 pg is seen and is very significant.

Discussion

The results herein complement previously reported efforts to enhance the expression of CD 34 and expansion of HSC [19]. HSCs undergo a massive increase in numbers *in vivo* during the process of hematopoietic reconstitution after stress, such as from infection, lipopolysaccharide challenge, chemotherapy, radiation, or transplantation [20,21], but this still cannot be recapitulated by *ex vivo* expansion approaches. Over the past decade, several novel studies have suggested that HSC expansion *ex vivo* might be feasible has led to a better understanding of specific factors regulating HSC self-renewal in vivo, and which have led to novel strategies to increase CD 34 enriched cells in *ex-vivo* [22,23]. Significant advances in HSC expansion have also been made recently in high-throughput screening approaches of low molecular weight compound libraries.Boitano et al. [11] used high-throughput screening of chemical compound libraries to identify compounds that afforded a molecule SR-1 an AHR antagonist which brought about an increase of CD34 expression ex vivo. Singh et al. [24] also showed that aryl hydrocarbon receptor has a role in the regulation of hematopoietic and other stem/progenitor cell and Metadichol an inverse agonist of AHR confirms this finding regulation of Stem Cells.

Also, Vitamin D supplementation also can significantly increase human UCB HSPCs in vitro. Cortes et al. [25] have pointed out how vitamin D signaling plays a role in HSPC expansion and survival in zebrafish embryos and human umbilical cord blood. They showed that HSPCs respond directly to [1,25] (OH)D3 stimulation via vitamin D receptor-induced transcriptional activation of the inflammatory cytokine CXCL8. VDR is expressed on human CD34 hematopoietic progenitors [26]. When it comes to bone marrow transplantations, patients can almost never find a donor whose HLA surface proteins match perfectly. However, if the HLA proteins of the donor are too different from those of the recipient, then the cells from the bone marrow transplant attack the recipient's cells and destroy them Graft versus Host Disease" (GVHD). The inability of leukemia and lymphoma and other patients to receive bone marrow transplants is the unavailability of matching bone marrow. Globally, patients are unable to get stem cell transplants needed to combat blood cancers such as leukemia because there is no donor mat Metadichol being an inverse agonist of both VDR and AHR can exploit multiple pathways in its actions on UBC.

Metadichol® has an advantage over other chemical entities. It is a food-based ingredient and is entirely devoid of side effects, unlike most synthetic molecules. It has the potential to restore a new functional hematopoietic system in recipients in cases where the patient is, for example, is leukemia. We have shown in two studies an MDS patient (see US patent 9,006,292) and a dengue case study where levels of Platelets and Neutrophils and WBC counts restored to normal levels [27]. Metadichol is uniquely positioned to exploit the power of expressing CD34 and its consequent differentiation and expansion that could be a significant step in offering to all patients who are in need of receiving transplants *ex vivo* or preferably as *in-vivo*.

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