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Expression of cell surface markers during self-renewal and differentiation of human adipose-derived stem cells

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ABSTRACT

Human adipose-derived stem cell populations express cell surface markers such as CD105, CD73, CD146 and CD140a/PDFGRα. However, it was unclear whether these markers could discriminate subpopulations of undifferentiated cells and whether the expression of these markers is modulated during differentiation. To address this issue, we analysed the immunophenotype of cultured human multipotent adipose derived stem (hMADS) cell populations at different adipocyte differentiation steps. We found that 100% of undifferentiated cells expressed CD73 and CD105. In contrast, CD146 and CD140a/PDFGRα marked two different subpopulations of cells. CD140a/PDGFRα subpopulation was regulated by FGF2, a critical factor of human adipose-derived stem cell self-renewal. During differentiation, CD73 was maintained and marked lipid-laden cells, whereas CD105 expression was inhibited in fully differentiated cells. The percentage of CD146 and CD140a/PDFGRα-positive cells declined as soon as cells had undergone differentiation. Altogether, these data support the notion that expanded adipose-derived stem cells are heterogeneous mixtures of cells and cell surface markers studied can discriminate subpopulations.

1. Introduction

The stromal vascular (SV) fraction of human white adipose tissues is a rich source of adipose-derived stem (ADS) cells, that are studied both by fundamental and clinical research specialists [1]. ADS cells are generally isolated from heterogeneous mixtures of SV cells by their capacity to adhere to plastic culture dishes. Then, due to their self-renewal ability, stem cells can be expanded in culture while maintaining the potential to undergo differentiation at least into adipocytes, osteoblasts and chondrocytes. However, these criteria only enable isolation of stem cells thereafter. ADS cells are described as expressing cell surface markers such as CD105, CD73, CD146 and CD140a/PDFGRα, which could be used for their prospective isolation. However, it is necessary to know whether these markers are expressed during the different steps of ADS maturation prior to isolation undifferentiated ADS cells from heterogeneous cell mixtures. To address this issue, we investigated cell surface marker expression during adipocyte differentiation of *in vitro* expanded human multipotent adipose-derived stem (hMADS) cell populations [2]. These stem cells have been isolated from white adipose tissues from young donors. They exhibit stem cell characteristics, i.e. the capacity to self-renew and differentiate into several cell types at the clonal level, even after long-term expansion. Expanded hMADS cells are able to differentiate under serum-free adipogenic conditions into cells displaying a combination of properties similar, if not identical, to those of native human adipocytes [3,4]. Altogether, hMADS cells appear to be a powerful cellular model to investigate cell surface marker expression during self-renewal and differentiation.

Fibroblast growth factor 2 (FGF2) has been reported to have a critical autocrine/paracrine role in human adipose-derived stem cell self-renewal. In addition, cells expanded by FGF2 have a high adipogenesis potential [5–7]. However, whether FGF2 regulates self-renewal of subpopulations of adipose-derived stem cells remains to be determined. We show in this study that hMADS cell populations are composed of subpopulations of cells expressing these cell surface markers at different levels. CD140a/PDGFRα marks a subpopulation of undifferentiated hMADS cells regulated by FGF2.

2. Materials and methods

2.1. Isolation and culture of human adipose progenitors

hMADS cells were obtained from the stroma of human adipose tissues as described previously [2]. Adipose tissue was collected,

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with the informed consent of the parents, as surgical scraps of surgical specimens from various surgeries, as approved by the Centre Hospitalier Universitaire de Nice Review Board. The cell populations that were studied here were isolated from the umbilical region fat pad of a 2-year old (hMADS1) female donor and from the prepubic region fat pad of a 4-month old (hMADS3) male donor. Human stroma vascular (SV) cells were isolated from subcutaneous adipose tissues from a 61 year-old female. Proliferation medium for routine maintenance of hMADS cells and SV cells was composed of DMEM (low glucose) containing 10% foetal calf serum, 10 mM HEPES, 100 U/ml penicillin and streptomycin. After reaching 80% confluence, adherent cells were dissociated in 0.25% trypsin EDTA. Cultures were maintained at 37 °C in a humidified gased incubator, 5% CO_2 in air.

2.2. hMADS cell and SV cell differentiation

hMADS cell adipocyte differentiation was performed as previously described [8]. Basically, confluent cells were cultured in DMEM/Ham's F12 media supplemented with transferrin (10 μg/ml), insulin (5 μg/ml), triiodothyronine (0.2 nM), dexamethasone (1 μM), isobutyl-methylxanthine (100 μM) and rosiglitazone (1 μg/ml). Three days later, the medium was changed (dexamethasone and isobutyl-methylxanthine were omitted). The SV cell adipogenic differentiation cocktail consisted of 10% FCS supplemented with insulin (5 μg/ml), triiodothyronine (0.2 nM), dexamethasone (0.25 μM), isobutyl-methylxanthine (500 μM) and rosiglitazone (1 μg/ml).

2.3. RT-PCR analysis

All primer sequences are detailed in supplemental Table 1. Real-time PCR assays were run on a StepOnePlus real-time PCR system (Applied Biosystems). Normalisation was performed using the geometric averages of the TBP housekeeping gene. Quantification was performed using the comparative-DCt method.

2.4. Flow cytometry analysis for cell surface markers

Cell surface antigens for hMADS cells were analysed by fluorescence-activated cell sorting (FACS) of living cells. We incubated 250,000 cells with each of the following conjugated human specific monoclonal antibodies: CD73 PE, CD105 FITC, CD146 FITC, PDGFRα/CD140a PE (BD Pharmingen). Nonspecific fluorescence was determined by incubation of similar cell aliquots with isotype-matched mouse monoclonal antibodies (BD Pharmingen). Data were analysed by collecting 10,000 events using a FACSCalibur flow cytometer with Cellquest acquisition software (BD Biosciences).

For CD73 and Lipidtox labelling, cells were incubated with HSC LipidTox Deep Red (Invitrogen, H34477), a neutral lipid stain and with CD73 PE antibody for 15 min at room temperature. Adipocytes were gated as previously described and the data were analysed by collecting 50,000 events using Cellquest acquisition software (BD Biosciences).

2.5. Statistical analysis

Statistical significance was determined by InStat3 software. Probability values <0.05 were considered as statistically significant. A nonparametric unpaired test (Mann–Whitney) was used.

3. Results and discussion

3.1. Expression of cell surface markers by undifferentiated and differentiating human adipose-derived stem cells

We analysed the expression kinetics of CD105, CD73, CD146 and CD140a/PDFGRα during the differentiation of two hMADS cell populations isolated from separate donors [2]. hMADS1 cells were isolated from the umbilical region fat pad of a 24-month old female donor and hMADS3 cells were isolated from the prepubic region of a 4-month old male donor. These cells populations were studied after 15–20 passages. As shown in Fig. 1A, the hMADS cell adipocyte differentiation process can be divided into two main states, i.e. the undifferentiated state corresponds to exponential cell growth phase or when cells are growth-arrested by cell–cell contact (which is determined as the day of differentiation induction). Around 7 days later, cells reached the terminal differentiated state when they accumulated lipid droplets. The expression of adipogenic genes, such as PPARγ2, FABP4 and leptin, was used to monitor the terminal differentiated state (Fig. 1B). Then cell surface marker expression during the differentiation process was determined by flow cytometry. As shown in Fig. 2, CD105 and CD73 were expressed by 90–100% of undifferentiated cells. CD106, CD34 and CD45 expression was not detectable (not shown). CD105 expression was dramatically reduced in differentiated cells, whereas CD73 expression was maintained during differentiation.

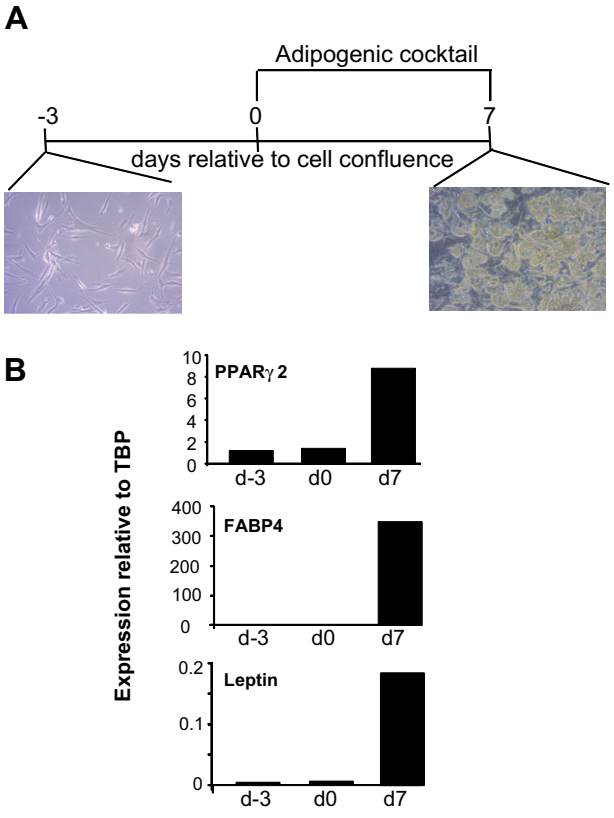

Fig. 1. Adipocyte differentiation of human adipose-derived stem cells. (A) Experimental scheme for differentiation of hMADS cells. Undifferentiated growing cells (day 3) were maintained in DMEM medium containing 10% FCS till the cells reached confluence (day 0). Then the cells were induced to undergo adipocyte differentiation in serum-free medium as described in the Materials and methods. Seven days later, cells were filled with lipid droplets. (B) Expression of adipogenic markers. RNAs were prepared at the indicated times and PPARg2, FABP4 and leptin expression was analysed by real-time PCR. The kinetics of expression is representative of several independent experiments.

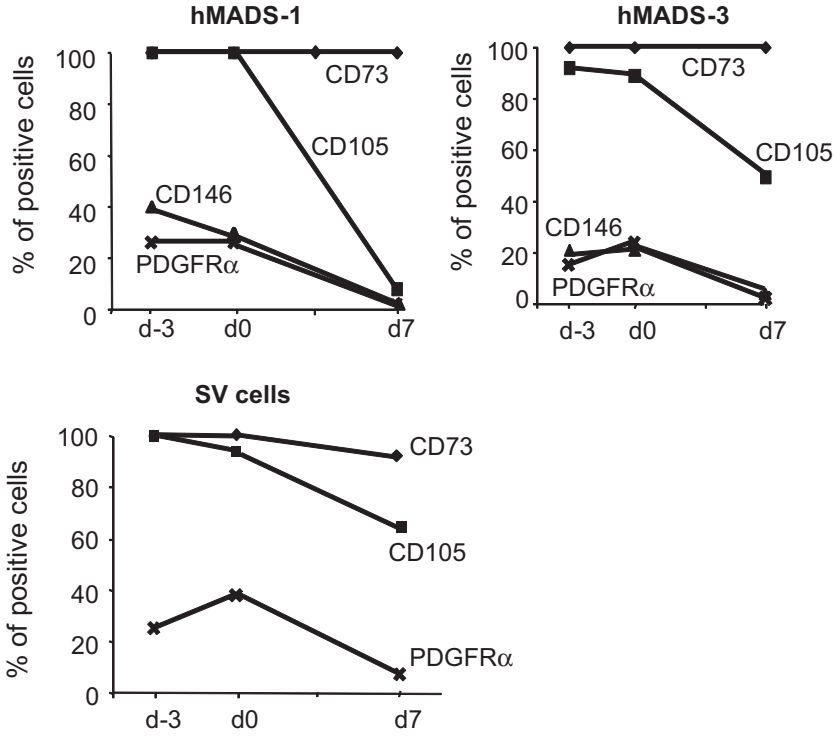

Fig. 2. Flow cytometry analysis of cell surface markers during adipocyte differentiation of hMADS cells and human adipose-derived stroma (SV) cells. hMADS1, hMADS3 and SV cells were stained at the indicated times for the surface antigens CD73, CD105, CD146 and CD140a/PDGFRα, and FACS analysis was performed. The *y* axis represents the percentage of positive cells.

To determine whether CD73 was also expressed in lipid-laden cells, cells were stained with Lipidtox, for triglyceride staining, and adipocytes were quantified by flow cytometry based on Lipidtox staining and granularity parameters, as previously described [9]. As shown in Fig. 3, CD73 marked both undifferentiated cells and adipocyte derivatives.

CD140a/PDGFRα and CD146 were expressed by a subpopulation of undifferentiated cells that represented around 20% of the total cell population. Then their abundance declined as soon as the cells had undergone differentiation. The abundance of CD140a/PDGFRα and CD146 subpopulations was 4 times lower in 7-day differentiated cultures than in undifferentiated ones ($n = 4$, $p < 0.05$). A similar expression profile was observed during differentiation of hMADS3 and hMADS1 expanded cell populations isolated from young donors and during differentiation of a primary culture of adipose tissue stroma cells isolated from an older subject (Fig. 2). These data indicated that CD140a/PDGFRα and CD146 allowed to distinguish two subpopulations of undifferentiated cells.

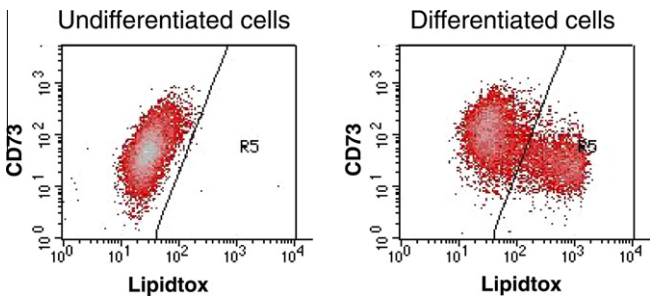

Fig. 3. CD73 marks both undifferentiated cells and mature adipocytes. Undifferentiated (day 0) and differentiated (day 7) cells were stained with Lipidtox and CD73 and then analysed by flow cytometry. This result is representative of 2 independent experiments.

Interestingly, the double positive CD140a/PDGFRα and CD146-positive cells represented a weak percentage of the undifferentiated culture (2%, not shown). The requirement of CD146 and of CD140a/PDGFRα inhibition for terminal adipogenic differentiation has yet to be demonstrated, but would be in agreement with the previously reported anti-adipogenic effect of PDGFα [10]. Altogether, these data highlighted that subpopulations of cells coexisted in undifferentiated hMADS cell populations. The properties of each subpopulation should be analysed in detail and the first step could be to investigate their regulation by factors that play a critical role in human adipocyte differentiation.

3.2. Regulation of cell surface markers by FGF2

We investigated factors regulating CD146 and CD140a/PDFGRα subpopulation abundance. First, we analysed whether component(s) involved in adipogenic commitment (see Materials and methods) might be involved in their down-regulation. hMADS cells were thus maintained in serum-free medium supplemented with transferrin and insulin and then exposed to dexamethasone (DEX) and IBMX for 3 days. Flow cytometry analysis revealed that a similar percentage of subpopulation was present in untreated and DEX-IBMX-treated cells (not shown). Then we investigated the effects of FGF2, i.e. a critical factor for stem cell maintenance. Interestingly, the percentage of CD140a/PDGFRα expressing cells dramatically increased upon FGF2 addition. As shown in Fig. 4, more than 80% ($n = 4$, $p < 0.05$) of the cells expressed CD140a/PDGFRα after 3 day stimulation with FGF2. In contrast, CD73, CD105 and CD146 subpopulations were not significantly changed. Inhibition of CD140a/PDGFRα expression during differentiation was maintained even when undifferentiated cells were treated with FGF2 (not shown). As we previously reported that hMADS cells secrete FGF2 [5], we analysed the role of the FGF2 autocrine/paracrine loop in the maintenance of a CD140a/PDGFRα-po-

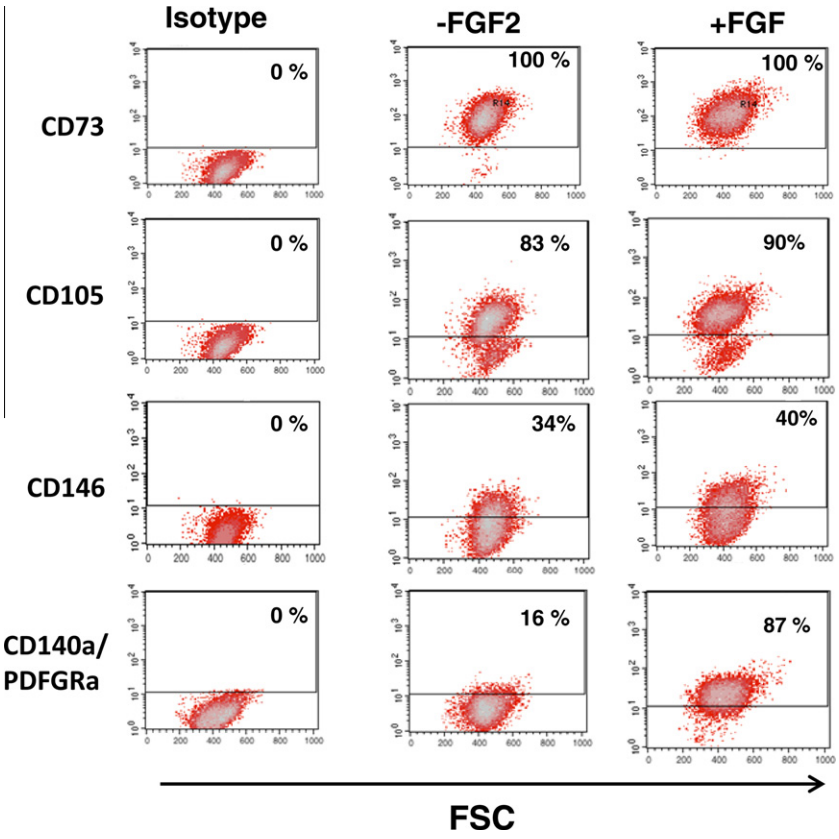

Fig. 4. Effects of FGF2 on the PDGFRα cell population percentage. hMADS3 cells were maintained in the absence or presence of 2.5 ng/ml FGF2 for 3 days. Cells were then stained for the indicated surface antigens. The *x*-axis represents the cell size and the *y*-axis indicates the relative fluorescence intensity from 10 to 10,000 on a logarithmic scale.

sitive cell population. In the absence of exogenous FGF2 addition, inhibition of the FGF pathway using PD173074, i.e. a specific FGF receptor inhibitor [11], led to a 70% ± 10% ($n = 2$) reduction in PDGFRα-positive cell abundance. These observations strongly suggest that the CD140a/PDGFRα cell sub-population was maintained mainly via endogenous FGF2 expression.

Altogether, these data indicate that CD140a/PDGFRα marks a subpopulation of cells in undifferentiated human adipose-derived stem cell populations. Interestingly, it has been reported, in a mouse muscle-derived cell population, that only the CD140a/PDGFRα cell population supports the adipogenic potential [12], as we also noted in humans (N. Clement and C. Dechesne, unpublished data). CD146 is described as a universal adult stem cell marker [13], but the CD146 cell population derived from adipose tissue does not display a high adipogenic potential [14]. To go further, it would be interesting to compare the adipogenic potential of CD146 and CD140a/PDGFRα cells and to more thoroughly investigate their self-renewal and the multipotency potential.

Our study revealed that expanded hMADS cells are heterogeneous populations, in agreement with the hierarchical model proposed for bone marrow-derived mesenchymal stem cell populations [15] and for a recent review see [16]). We suggest that the CD140a/PDGFRα cell subpopulation could represent a core of immature cells that is maintained by the FGF2 pathway.

Acknowledgments

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