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Epithelial differentiation of human adipose tissue-derived adult stem cells

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Abstract

Adult human stem cells are employed in novel treatments and bio-artificial devices. Recent studies have identified an abundant source of stem cells termed adipose-derived adult stem (ADAS)-cells in the subcutaneous adipose tissue. Under appropriate culture conditions ADAS-cells differentiate to various cell types, including chondrocytes, adipocytes, and smooth muscle cells. Aiming at epithelial differentiation this study investigated the effect of all-*trans* retinoic acid (ATRA) on human ADAS-cells. ATRA-induced cytokeratin 18 expression in ADAS-cells and nearly abolished vimentin expression as shown by Western blot. In immunofluorescence, the formation of keratin fibers in ATRA-treated ADAS-cells could be observed. The percentage of ADAS-cells being able to undergo epithelial differentiation as quantified by FACS-analysis was above 80%. Inhibition of cell growth by ATRA was shown using DAPI- and MTT-assays. ATRA can differentiate ADAS-cells toward the epithelial lineage. This finding, along with a previously described neural differentiation, shows that ADAS-cells have epithelial potential.

Keywords: Stem cells; Mesenchymal; Differentiation; Epithelial cells; Lipoaspirate; Tissue engineering; Bio-artificial renal assist device; Retinoids; ATRA; Cytokeratin

Stem cells in human adipose tissue, isolated from lipoaspirates, have proven their capacity to differentiate in vitro. These cells are a subpopulation of the non-adipocyte cell fraction in lipoaspirates, the so-called stroma vascular fraction (SVF). The SVF-cells, unlike the adipocytes, sediment in aqueous medium and a subset of these cells attaches to and grows on tissue culture plastic. Zuk et al. [1] have shown that these adherent cells have surface antigens and differentiation potential similar to those of mesenchymal stem cells (MSC) from human bone marrow stroma. In vivo the cells function as adipocyte precursor cells forming new adipocytes when the total mass of an organism's adipose tissue is increased [2,3]. The common terminology used for these adipose-derived cells is processed lipoaspirate (PLA) cells or ADAS (adipose-derived adult stem) cells [4].

ADAS-cells can be procured easily from the donor, which makes the vision of clinical application more feasible. Further these cells are derived from adults and therefore circumvent the ethical ambiguities of using embryonic stem cells. Once obtained and then modified in vitro, these cells can be reintroduced autologously, which has the advantage of reducing the probability of an immune reaction.

Significant attempts are presently directed to harvesting the characteristics of stem cells of various origin for developing novel treatments. Such stem cells may be useful in acute and chronic renal injury for the replacement of damaged nephrons [5]. Until recently, successful attempts have mainly been performed using stem cells of hematopoietic origin [6–9]. Now it has been shown in a mouse model that mesenchymal stem cells can engraft in

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the kidney with acute renal failure and improve nephron regeneration [10]. Even a native stem cell population has finally been identified in the kidney of mice and rats which seems to play a significant role in renal repair after transient ischemia [11–13].

Stem cells may also be used in the development of renal replacement technologies for exogenous filtration. Clinical trials are already evaluating renal tubule assist devices containing renal tubule cells in series with conventional hemofiltration cartridges [14,15]. Considering that both tissues have the same mesenchymal origin in embryonal organogenesis, we hypothesize that ADAS-cells may serve as an alternative for renal cells in a renal assist device, once differentiated toward the epithelial lineage.

Our interest in studying retinoids is based on the observation that they act as inducers of cell differentiation in many organs and can influence the expression of intermediate filaments such as keratins [16–20]. In the present study, we analyzed the effects of ATRA (all-*trans* retinoic acid) on ADAS-cell differentiation with the goal to differentiate epithelial cells. We tried to answer the following questions: Are ADAS-cells sensitive to ATRA and which changes does ATRA induce in ADAS-cells? Taken together, the results demonstrated that: (1) ADAS-cells are sensitive for ATRA, and (2) ATRA induces epithelial differentiation of ADAS-cells which undergo morphological changes, form monolayers, and change their protein expression profile.

Methods

Cell isolation and culture. Lipoaspirates from patients undergoing cosmetic liposuction were processed taking pattern from methods originally described by Björntorp and later modified by Hauner for preadipocyte isolation [2,3,21]. Zuk et al. [1] showed that this method is capable of providing a population of multipotent mesenchymal stem cells.

Briefly the tissue was digested for 45 min with 0.075% collagenase type 1. The stromal–vascular fraction was then separated from remaining fibrous material and the floating adipocytes by centrifugation at 300*g*. The sedimented SVF-cells were filtered through a 100 μm pore filter followed by an incubation step in an erythrocyte lysing buffer (160 mM NH_4Cl) before fluorescence activated cell sorter (FACS) analysis. For cell culture erythrocyte contamination was reduced by density gradient centrifugation with Biocoll (Biochrom AG, www.biochrom.de) instead. The high contamination with erythrocytes was found to markedly decrease cell adherence and proliferation. A preceding density gradient separation provided a better yield of adherent cells than treatment with an erythrocyte lysing buffer.

For initial cell culture and expansion of the SVF-cells DMEM with a physiologic glucose concentration (1000 mg/L) supplemented with 10% fetal calf serum (FCS) was used. The cells were passaged to chambered slides for immunofluorescence assays or cultured in flasks for FACS analysis, proliferation and viability assays were carried out in 96-well plates (Nalge Nunc International, www.nalgenunc.com).

Adipogenic differentiation protocol. For characterization purposes of isolated ADAS-cells an adipose cell differentiation protocol according to Zuk et al. [1] was carried out. The adipogenic differentiation medium consisted of DMEM, supplemented with 10% fetal bovine serum, 0.5 mM isobutyl-methylxanthine, 1 μM dexamethasone, 10 μM insulin, and 200 μM indomethacin (Sigma, www.sigmaaldrich.com). After 20 days the induced ADAS-cells were stained with Oil Red O (Sigma) for lipid detection.

Epithelial differentiation protocol. For epithelial differentiation the cells were incubated with ATRA (Sigma) at a final concentration of 5 μM. This concentration was determined by taking pattern from previous studies and by testing with proliferation and vitality assays described later.

A stock solution of ATRA dissolved in dimethyl sulfoxide (DMSO) at 10 mM was kept at −80 °C. For cell culture ATRA was dissolved in low glucose (1000 mg/L) DMEM substituted with 10% fetal bovine serum (FBS). The equivalent volume of solvent (DMSO) without ATRA was used in control samples. The medium was replaced every two days during a total incubation period of 10 days followed by analysis of the resulting cell population.

Flow cytometry. Flow cytometric characterization of freshly isolated SVF-cells, undifferentiated ADAS-cells ranging from passage one to seven, or differentiated ADAS-cells was performed. The resuspended cells were fixed in PBS containing 1% formaldehyde, resuspended in PBS containing 0.5% BSA, 5.3 mM EDTA, and 0.03% saponin as required, and distributed to 10^6 cells per sample. These aliquots were incubated with primary antibodies for 30 min on ice and in the presence of saponin when staining intracellular antigens. Antibodies, unconjugated or conjugated with either FITC, PE or Cy5, directed against the following antigens were employed: CD2 (FITC, Becton–Dickinson, www.bectondickinson.com), CD3 (PE, Becton–Dickinson), CD10 (PE, BD Biosciences PharMingen, www.bdbiosciences.com/pharmingen), CD11a (FITC, Immunotools, www.immunotools.com), CD13 (FITC, Immunotools), CD14 (PE-Cy5, Beckman Coulter, www.beckmancoulter.com), CD31 (primary, DakoCytomation, www.dakocytomation.com), CD34 (primary, Immunotech), CD44 (FITC, Immunotools), CD45 (PE, Immunotools), CD90 (primary, Sigma), and Cytokeratin 18 (primary, Dako).

After washing, the cells were incubated with a FITC-labeled secondary antibody (Goat anti-mouse IgG, Dianova, www.dianova.com) as required. A propidiumiodide (Sigma) staining was performed (1 μg/mL), especially with freshly isolated SVF cells, to discern nucleus containing cell populations from erythrocytes and debris.

The cytometer was a FACScalibur (Becton–Dickinson) with a 488 nm krypton–argon laser. For data analysis samples were compared with isotype-matched controls.

Immunofluorescence. For immunofluorescence staining the cells were cultured in 8-well chambered slides (Nunc). The cells were fixed with ice-cold acetone/methanol (1:1) and washed with PBS. For blocking unspecific binding sites the cells were incubated with PBS containing 5% normal goat serum and human IgG (5 mg/mL) for 20 min. Next primary antibodies were employed at 4–10 μg/mL for 45 min on ice. Antibodies directed against the following human antigens were used: Thy-1 (CD90, Sigma), pan-cytokeratin (clones AE1 & AE3, Dako), cytokeratin 10/13 (Dako), cytokeratin 18 (Dako), vWF (von Willebrand factor, Dako), and vimentin (Sigma). A Cy3-labeled secondary antibody mouse (goat anti-mouse IgG + IgM mAb, Dianova) was then used at a concentration of 1.4 μg/mL. Further a DAPI (4,6-diamidino-2-phenylindole dihydrochloride) nuclear stain was performed (0.5 μg/mL). All dilutions of antibodies were made in PBS containing 1% goat serum and human IgG (1 mg/mL). Isotype-matched primary antibodies served as controls. The slides were mounted in glycerol/gelatin and examined using Zeiss' fluorescence microscope equipment.

Western blot. For Western blot analysis the cells were washed twice with ice-cold PBS and lysed using 10 mM Tris, pH 7.4, 0.1% SDS, 0.1% Tween 20, 0.5% Triton X-100, 150 mM NaCl, 10 mM EDTA, 1 M urea, 10 mM NEM, 4 mM benzamidine, and 1 mM PMSF. The resulting lysate was collected by scraping and centrifugation, and the protein concentration in the supernatant was determined using Lowry's method. Five micrograms of each sample was diluted in Laemmli's

buffer and heated at 95 °C for 5 min prior to electrophoresis on a 10% SDS–polyacrylamide gel. The separated proteins were electrophoretically transferred to Immobilon transfer membranes (Millipore, www.millipore.com). The membranes were incubated overnight in blocking buffer (10 mM Tris-buffered saline at pH 8, 0.1% Tween 20, and 3% nonfat powdered milk) in order to block non-specific binding sites. The primary antibody, either anti-cytokeratin 18 (DAKO) or anti-vimentin (Sigma), was added to the membranes, and incubated for 1.5 h. After three washes, the secondary antibody was added (horseradish peroxidase-conjugated anti-mouse IgG, Amersham, www.amersham.com) for 1 h. Protein bands were made visible using an enhanced chemiluminescence system (Amersham) and were recorded on radiographic film. Densitometric analysis was performed with ImageJ (NIH, www.nih.gov).

Proliferation and viability assays. Proliferation was measured with a non-radioactive fluorometric assay with the DNA-intercalating fluorochrome DAPI (Sigma) [22]. Six thousand cells per well, cultured in a 96-well plate, were incubated for 48 and 96 h with ATRA ranging in concentration from 0.5 to 100 μM. The effect of DMSO as solvent for ATRA was controlled. After the incubation period the cells were washed with PBS, and lysed using 0.02% (w/v) SDS, 150 mM NaCl, and 15 mM sodium citrate. Finally, DAPI (2 μg/mL) was added to each well and relative DNA content as compared to the negative control was measured in a fluocytometer (FLUOstar, BMG Lab-Technologies, www.bmg-labtechnologies.com). A 355 nm excitation filter and a 460 nm emission filter were used, and data were acquired as arbitrary light units.

Viability was assessed using a classic MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay with the same incubation protocol as the proliferation assay. After the incubation period the cells were incubated with MTT (50 μg/well) in PBS for 4 h and then treated with 0.04 M HCl/2-propanol. MTT metabolized into formazan was measured with a photometer at 560 nm.

Data analysis and statistical testing. The data were analyzed using non-parametric methods. Significance was defined as $p < 0.05$ using Wilcoxon's test for sample comparison.

Results

Characterization of cells from human lipoaspirate

The freshly isolated SVF cells were analyzed with a flow cytometer in order to characterize the cellular composition and possible contaminants of the stem cell culture. We found a characteristic set of cell populations that could be roughly distinguished by cell size and granularity in forward and sideward scatter diagrams. Propidiumiodide positive cell populations (cells with a nucleus) found in the SVF were granulocytes (CD14+, CD11a+, and CD45+, high granularity), endothelial cells (CD13+, CD31+, CD34+, and CD90+), lymphocytes (CD2+, CD3+, and CD45+), and monocytes/macrophages (CD13+, CD14+, CD31+, and CD45+). Sharing membrane antigens with other cells found in the SVF (CD10, CD13, CD44, and CD90), an ADAS-cell population could not be clearly distinguished. Still it is best characterized by the surface marker Thy-1 (CD90), only sharing this feature with endothelial cells (e.g., Fig. 1). SVF cell composition, characterized by surface antigens, is shown in Table 1 as median portion of positive cells from triplicate isolations.

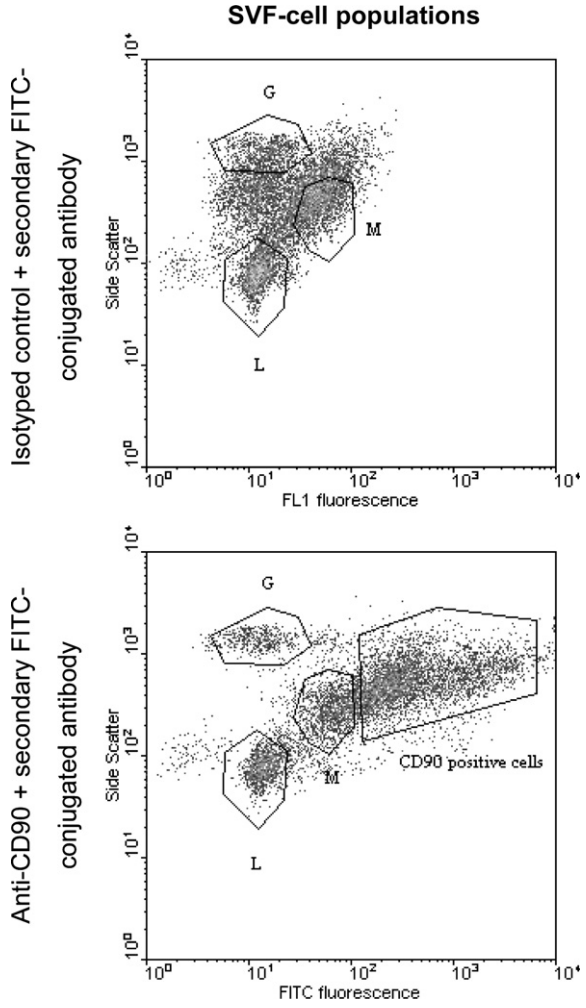

Fig. 1. SVF-cells contain a population of CD90 positive cells, a surface marker that cultured ADAS-cells share with endothelial cells. The figure shows density plots of SVF-cells (fluorescence 1 against sideward scatter) stained with anti-CD90 and a secondary FITC-conjugated antibody, and the appropriate control, respectively. Various other populations characterized as macrophages (M), granulocytes (G), and lymphocytes (L) are indicated.

Table 1
Composition of SVF (stroma vascular fraction)-cells and characterization of ADAS (adipose-derived adult stem)-cells

Marker	Portion of positive cells in		Cell type
	ADAS (%)	SVF (%)	
PI	100	100	Non-erythrocyte cells with nucleus
CD2	0	11	Lymphocytes
CD11a	0	18	Granulocytes
CD14	0	29	Macrophages, granulocytes
CD31	0	49	Endothelial cells, macrophages
CD34	0	55	Macrophages, endothelial cells
CD45	0	57	Granulocytes, macrophages, and lymphocytes
CD90	100	60	ADAS-cells, endothelial cells

The table shows the median portion of cells from triplicate isolations positive for the listed surface antigens. The cell type that is assumed to represent this fraction is stated.

The fraction of adherent cells, when cultured on tissue culture plastic with DMEM and 10% FCS, is considered as multipotent ADAS-cells. When analyzing these adherent cells with a flow cytometer none of the cell populations mentioned above, namely macrophages, endothelial cells, lymphocytes, and granulocytes, remained. Especially the presence of endothelial cells, which can also be cultured as adherent cells in the presence of FGF and VEGF, and macrophages/monocytes, was eliminated. The ADAS-cells cultured in our laboratory were characterized by immunofluorescence and flow cytometry as CD10+, CD13+, CD31−, CD34 −, CD44+, CD45−, and CD90+, vimentin+, vWF− (von Willebrand factor). The differentiation potential of these cells was verified by performing the adipose cell differentiation protocol. This resulted in the accumulation of intracellular lipid droplets that could be stained with Oil Red O. Non-induced ADAS-cells showed no Oil Red O staining.

Morphological changes of differentiated ADAS-cells

For the differentiation of ADAS-cells toward the epithelial lineage the cells were cultured with 5 μM ATRA for 10 days. The effect of ATRA could be observed with phase-contrast microscopy. As documented in Fig. 2 the cells incubated in the presence of ATRA presented growth characteristics more resembling a monolayer culture of epithelial cells. In the control culture of non-induced ADAS-cells growth of cells in multiple layers was commonly observed, the latter being typical for mesenchymal cell cultures.

Epithelial marker expression in differentiated ADAS-cells

Immunofluorescence was used to investigate changes of phenotype after the differentiation of ADAS-cells with 5 μM ATRA for 10 days. We found that ATRA initiated the expression of cytokeratin 18 in ADAS-cells. As documented in Fig. 3 the immunofluorescence stains with anti-cytokeratin 18 presented a filamentous cytoskeleton, the typical appearance of epithelial cytokeratins. Other cytokeratins as investigated with a pan-cytokeratin antibody mix probing for cytokeratins with Moll's designation 1–8, 10, 13, 14–16, and 19 were not expressed. The controls showed no immunofluorescence staining of cytokeratins. Vimentin filaments were present in both, controls and ATRA-stimulated cells.

Western blot analysis was performed for quantitative analysis of differentiation marker expression. As shown in Fig. 4, ATRA increased the production of cytokeratin 18 and decreased the production of vimentin in ADAS-cells. In contrast to immunofluorescence analysis, Western blot analysis showed a marginal expression of cytokeratin 18 in unstimulated control cells. Those cells were cultured for at least 2 passages. This effect was reversible when inducing adipocyte differentiation in these ADAS-cells with an adipogenic medium. Freshly isolated and plated ADAS-cells cultured for only 6 days showed no expression of keratins.

Quantification of cells undergoing epithelial differentiation

FACS analysis was used to calculate the relative number of cytokeratin 18 positive ADAS-cells after

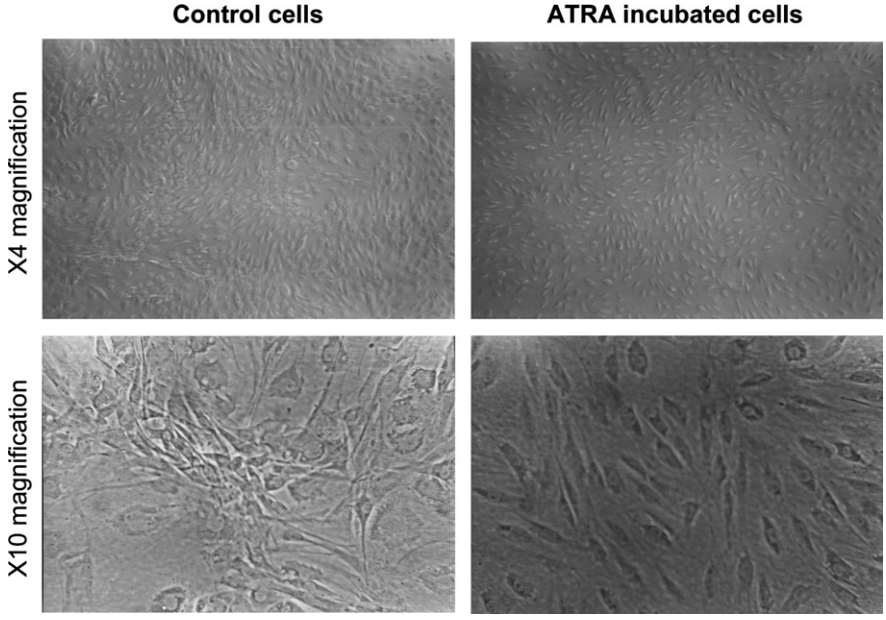

Fig. 2. Growth characteristics of ADAS-cells on tissue culture plastic as documented with phase contrast microscopy. This figure shows two magnifications of cells grown in control medium (left column) and in ATRA supplemented medium (right column). ADAS-cells incubated with ATRA for 10 days grow in monolayer culture, whereas the control cells grow in multiple layers.

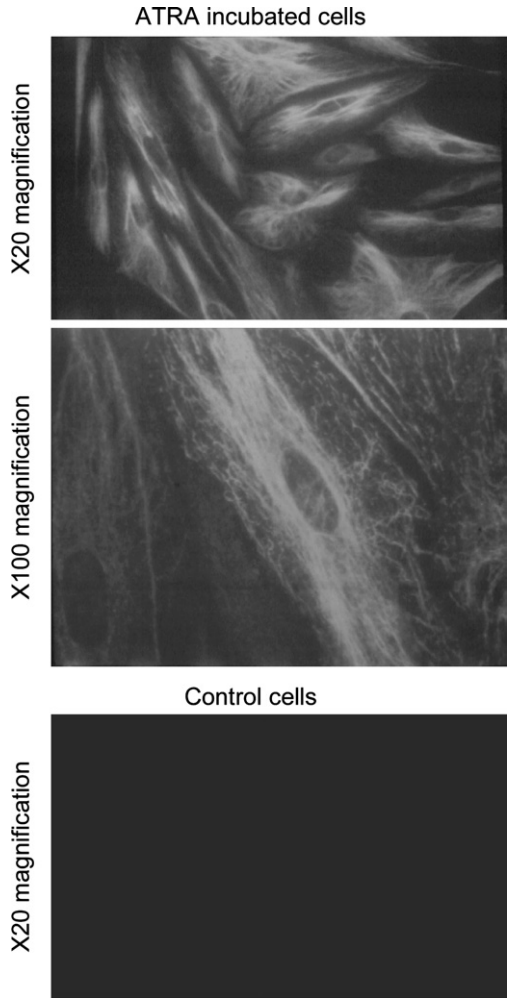

Fig. 3. ADAS-cells incubated with ATRA express cytokeratin 18. This figure shows immunofluorescence of ADAS-cells stained with anti-cytokeratin 18 after incubation of these cells with ATRA for 10 days. The magnification steps present a cytoskeleton typical in appearance for cytokeratin fibers in epithelial cells. A negative staining of undifferentiated control cells is included.

stimulation with ATRA for 10 days. Our results showed that at least 80% of the cells were definitively positive for cytokeratin 18 with the whole cell population being shifted away from the control's fluorescence levels (e.g., Fig. 5). Samples have proven to be significantly different as calculated with Wilcoxon's rank sum test.

Effect of ATRA on cell proliferation and viability

Proliferation and viability were investigated after 48 and 96 h of incubation. Measurements after 48 h of incubation showed no significant effect on cell proliferation (data not shown), after 96 h, however, even lowest concentrations of ATRA developed a perceivable anti-proliferative effect. Remarkably this effect was not concentration dependent, but was constant over the tested concentration range, with relative cell growth reduced to approximately 80% as measured by the DAPI proliferation assay (e.g., Fig. 6). Cell viability, however, was reduced by ATRA in a concentration dependent manner after 48 (not shown) and 96 h. It has to be taken into consideration that vitality was biased in ATRA samples by reduced cell number as shown with the proliferation assay. Further DMSO was considerably affecting viability in higher concentrations. Still, high concentrations of ATRA exerted a strong cytotoxic effect that cannot be attributed to anti-proliferative effects or DMSO toxicity. At the concentration we used for differentiation of the cells (5 μM ATRA) DMSO toxicity did not occur and vitality of the ADAS-cells was reduced to 70% (Fig. 7). These results served as our rationale for using a final concentration of 5 μM ATRA in the differentiation experiments.

Discussion

Just as bone marrow-derived adult stem cells the mesenchymal stem cells in human adipose tissue are defined

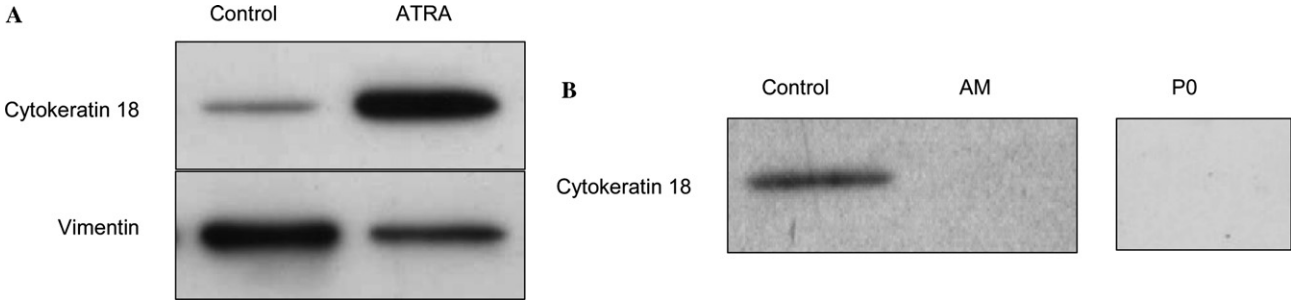

Fig. 4. Western blot showing (A) the effect of ATRA on the expression of the differentiation markers vimentin and cytokeratin 18. ADAS-cells were incubated with ATRA according to the differentiation protocol. The ATRA-treated cells show an increase in cytokeratin 18 expression and a reduced expression of vimentin. Densitometry of this representative blot shows a 5.2-fold increase in cytokeratin 18- and a 2.2-fold decrease in vimentin expression. (B) A marginal expression of cytokeratin 18 was observed in control cells not stimulated with ATRA. This occurred after the cells were grown and passaged, whereas freshly isolated unpassaged cells after 6 days of culture (P0) did not show cytokeratin 18 expression. Further this low level expression of cytokeratin 18 in control cells was reversible when adipocyte differentiation was induced using an adipogenic medium (AM).

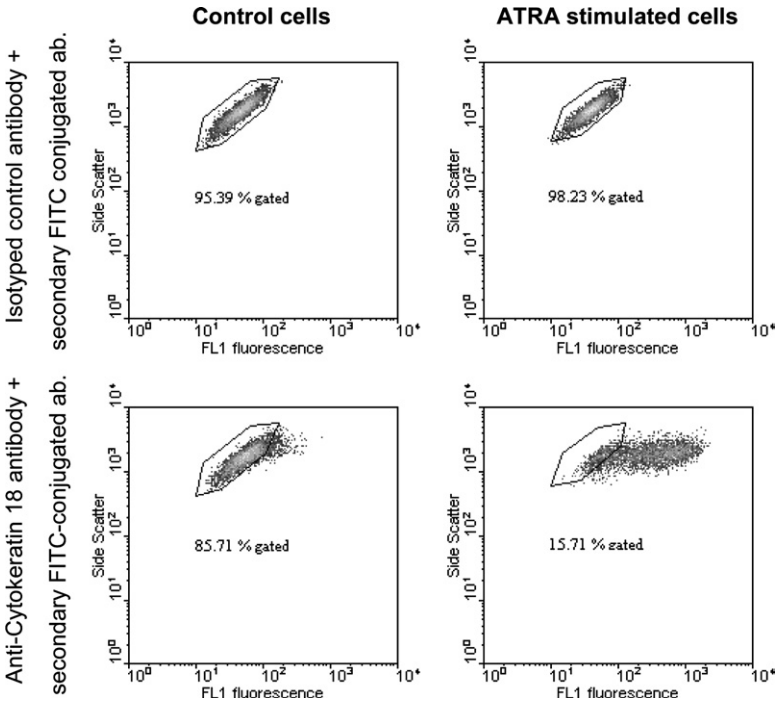

Fig. 5. ATRA incubated ADAS-cells express cytokeratin 18 as shown by quantification with flow cytometry. The figure shows density blots of ADAS-cell populations previously incubated with ATRA and the appropriate control cells incubated in control medium. Fl-1 signal strength displays intracellular cytokeratin 18 stained with an anti-cytokeratin 18 antibody and a FITC-conjugated secondary antibody. Propidiumiodide was used as a nuclear stain to discern the positive cells from negative debris. A polygonal region, identical for sample and control, was manually set up to evaluate the percentage of cells with intracellular cytokeratin 18 as compared to the control.

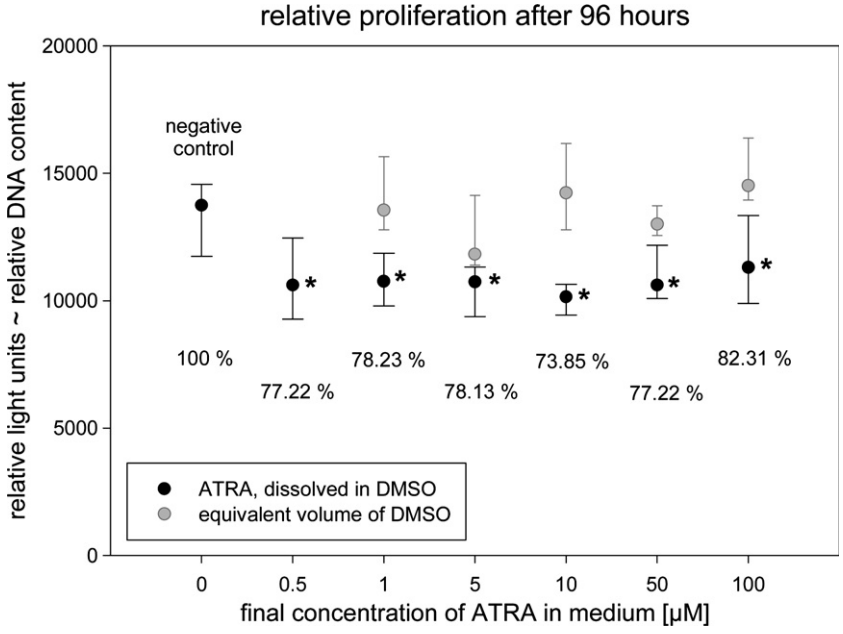

Fig. 6. ATRA inhibits ADAS-cell proliferation over a wide concentration range. A DAPI assay was used to measure DNA content as arbitrary light units in a fluocytometer. This figure shows DNA content of samples of ADAS-cells incubated with ATRA at various concentrations for 96 h. As a control the effect of DMSO, which is used as solvent in the ATRA stock solution, is displayed. The data are shown as sample medians with their 25% (upper bar) and 75% (lower bar) percentiles. Below the DNA content relative to the negative control is displayed. An asterisk indicates samples significantly differing from the negative control.

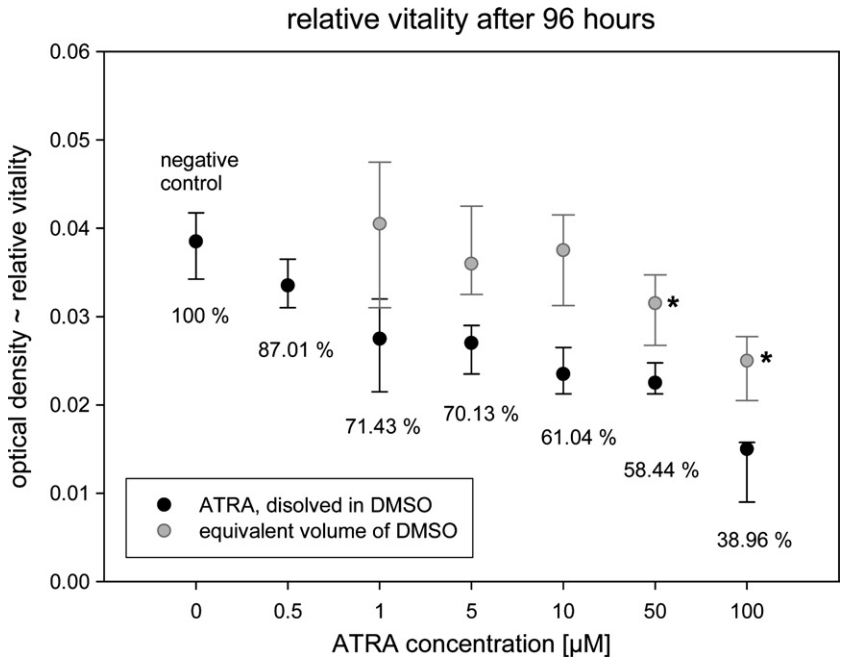

Fig. 7. ATRA has a concentration dependent impact on cell viability as measured with the MTT-test. The data are shown in analogy to Fig. 6, here with viability relative to the negative control below the samples. Wilcoxon's test has only been performed with DMSO samples due to the proliferation bias (see Fig. 6) of ATRA-treated samples.

by their ability to self-replicate and differentiate toward different cell lineages. However, this characteristic is only revealed after differentiation and, although surface antigen characterization has been extensively performed, no specific marker is known so far that would allow a preemptive and unequivocal identification of these stem cells [1,23,24].

With our characterization of uncultured, freshly isolated SVF-cells we showed that most of the known markers for ADAS-cells are abundant in the stroma vascular fraction from human lipoaspirates. Cells as macrophages and endothelial cells share common ADAS-cell antigens, such as CD13 or CD44. Anyway, we have shown that Thy-1 (CD90) is a relatively distinct antigen which ADAS-cells share with endothelial cells. This might prove useful in the attempt to selectively isolate these cells, for instance by immunomagnetic means. However, it is still in question whether ADAS-cells are a homogeneous population of cells by themselves or, as has been shown for marrow-derived mesenchymal stem cells [25,26], represent a heterogeneous cell population consisting of several different cell types.

In the field of in vitro mesenchymal stem cell differentiation a variety of substances have been employed to induce differentiation. The majority of these differentiate the stem cells into mesodermal derivatives such as smooth muscle cells or cartilage cells [4,27,28]. Recently, neural differentiations of marrow-derived mesenchymal stem cells have been reported [29,30] and the techniques used there could successfully be transferred on adipose-derived mesenchymal stem cells. Neurogenic induction of ADAS-cells resulted in a neuronal morphology and the expression of neuron-specific enolase (NSE) and neuron nuclei protein (NeuN), which are neuron-specific proteins [1,31]. This suggests an epithelial capacity of ADAS-cells. We speculated that retinoids might be used to induce epithelial differentiation, since retinoid receptors are known to display a strong differentiative and anti-proliferative activity in various tissues [32]. In this study, the effect of ATRA on ADAS-cells was investigated. Our proliferation and viability studies with ATRA showed that a concentration of 5 μM had the expected inhibitory effect on cell proliferation with a tolerable impact on cell viability. Further experiments revealed that ADAS-cells can potentially enter the epithelial lineage when treated with retinoids. The differentiated ADAS-cells reduced the expression of the mesenchymal marker vimentin and started the expression of an epithelial cytokeratin, more exactly cytokeratin 18.

Keratin filament proteins are one of the first epithelial-specific structural proteins to be synthesized in a differentiation program. In differentiating epithelia, keratin expression proceeds through the initial expression of "primary" keratins, supplemented later by secondary keratins. Unlike stratified epithelia, simple epithelia, such as glandular, intestinal or kidney epithelia, express solely their primary keratins, as, for instance, cytokeratin 18. They may acquire the expression of additional simple epithelial keratins (cytokeratin 7, 19, and 20) as

their differentiation progresses [33]. Among keratin proteins cytokeratin 18 is one of the oldest and most highly conserved keratins and is potentially playing an important role in cell polarization.

A marginal expression of cytokeratin 18 was also detected in non-induced ADAS-cells by Western blot analysis. This effect might have contributed to the polarizing tissue culture conditions on plastic surfaces which favor monolayer growth and present a surface charge. Such conditions could induce the discrete changes in marker expression that were observed. Another possible reason for spontaneous cytokeratin 18 expression in ADAS-cells is vitamin A in the bovine serum supplements. Although highly unstable retinoids have biological effect in small concentration so that vitamin A remnants in control media could be responsible. A possible workaround might be to employ three-dimensional tissue culture systems such as Matrigel or fibrin gels and serum-free media. However, we found that ADAS-cells do not proliferate and are difficult to maintain under serum-free conditions. Further an appropriate growth factor mix, which can be used to hold ADAS-cells in undifferentiated state, has still got to be defined. These particular observations lead to a more general limitation of in vitro culture of ADAS-cells: the cultured cells lack the specific environment they have in vivo which consists mainly of various growth factors, hormones, and cytokines, and the extracellular matrix in adipose tissue. Nevertheless, the marginal expression of cytokeratin 18 was reversible when the cells were exposed to an adipocyte differentiation inducing medium. The cells underwent morphologic changes with lipid droplet formation and stopped cytokeratin 18 expression.

In summary, the expression of the early cytokeratin 18 by ADAS-cells in combination with decreased vimentin expression must be considered as a first step toward epithelial differentiation. The cells share cytokeratin 18 with other simple epithelia such as liver and especially kidney epithelial cells. Kidney epithelial cells which are isolated as proximal and distal tubule cells from adult human kidneys express this particular cytokeratin under cell culture conditions. In future experiments, we will investigate how ADAS-cells can be differentiated fully to renal epithelial cells, taking up functional characteristics such as vitamin D production.

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