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Comparative gene array analysis of progenitor cells from human paired deep neck and subcutaneous adipose tissue

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ABSTRACT

Brown and white adipocytes have been shown to derive from different progenitors. In this study we sought to clarify the molecular differences between human brown and white adipocyte progenitors cells. To this end, we performed comparative gene array analysis on progenitor cells isolated from paired biopsies of deep and subcutaneous neck adipose tissue from individuals ($n = 6$) undergoing neck surgery.

Compared with subcutaneous neck progenitors, cells from the deep neck adipose tissue displayed marked differences in gene expression pattern, including 355 differentially regulated (>1.5 fold) genes. Analysis of highest regulated genes revealed that *STMN2, MME, ODZ2, NRN1* and *IL13RA2* genes were specifically expressed in white progenitor cells, whereas expression of *LRRC17, CNTNAP3, CD34, RGS7BP* and *ADH1B* marked brown progenitor cells.

In conclusion, progenitors from deep neck and subcutaneous neck adipose tissue are characterized by a distinct molecular signature, giving rise to either brown or white adipocytes. The newly identified markers may provide potential pharmacological targets facilitating brown adipogenesis.

1. Introduction

Brown adipose tissue (BAT) is able to maintain body temperature by non-shivering thermogenesis, a process driven by the dissipation of the proton motive force in the mitochondrial respiratory chain mediated by the uncoupling protein 1 (UCP1) (for review see Cannon and Nedergaard, 2004). The recent discovery of BAT activity in human adults (Nedergaard et al., 2007) reignited interest in its usage as a therapeutic tool against obesity (Nedergaard and Cannon, 2010; Tam et al., 2012). In three studies in 2009, symmetric areas of high glucose uptake in the neck detected by PET/CT scans in adults were identified as metabolically active BAT (Cypess et al., 2009; Van Marken Lichtenbelt et al., 2009; Virtanen et al., 2009). Most interestingly, the appearance and activity correlated with BMI (Saito et al., 2009; Zingaretti et al., 2009) indicating a potential role of BAT in the regulation of body weight.

Lineage tracing experiments performed in mice revealed that white adipocytes originate from different progenitors than brown adipocytes in the interscapular brown fat depot (Cypess et al., 2013; Lidell et al., 2013; Saito et al., 2009; Wu et al., 2012). Moreover, another intermediate brown-like cell phenotype arising in white adipose tissue – called "beige" adipocyte – can be distinguished from classical brown adipocytes by marker gene analysis (Wu et al., 2012). Recently, two studies in humans showed that the appearance of brown adipocytes is strongly dependent on the adipose tissue depot and that marker expression in these mature adipocytes is different from rodent models (Cypess et al., 2013; Lidell et al., 2013).

Progenitor cells from human brown adipose tissue have been isolated recently from different regions in the neck. Interestingly, differentiating these cells *ex vivo* revealed molecular and functional differences, which strongly depends on the location of adipose

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Table 1
Characteristics of patients included in this study. Paired biopsies from $n = 26$ for immunohistochemistry (A), $n = 14$ patients for tissue RNA (B) and $n = 12$ patients for isolation of progenitor cells (C) undergoing neck surgery were obtained. $n = 3$ patients were overlapping in group B and C and $n = 4$ patients in group A and C. Values are displayed as means ± SD.

	Total $n = 45$ (24m, 21f)	A $n = 26$ (14m, 12f)	B $n = 14$ (6m, 8f)	C $n = 12$ (8m, 4f)
Age (years)	55 ± 16 [18–79]	56 ± 15 [18–79]	58 ± 17 [23–77]	48 ± 18 [18–68]
BMI (kg/m^2)	27.1 ± 5.0 [18.0–39.6]	26.8 ± 4.4 [19.0–34.9]	28.9 ± 6.7 [18.0–39.6]	27.3 ± 5.3 [22.2–39.6]
fT_3 (pmol/l)	5.05 ± 0.78 [3.3–7.1]	5.04 ± 0.78 [3.3–7.1]	5.02 ± 0.81 [3.4–6.5]	5.23 ± 1.04 [3.8–7.1]

tissue (Cypess et al., 2013; Jespersen et al., 2013; Lee et al., 2011). This demonstrates that different pools of precursor cells exist in different white and brown adipose tissue depots. The molecular signature of human white and brown adipocyte progenitor cells has not been determined so far.

This study aimed at identifying novel markers of human brown adipocyte precursor cells. Thus, progenitor cells from paired samples of deep neck and subcutaneous neck adipose tissue were isolated, and gene expression patterns were analyzed using gene array analysis.

2. Materials and methods

2.1. Subjects

Primary adipose tissue samples were obtained from patients undergoing neck surgery for malignancies or nodular goiter. Deep neck adipose tissue was taken from the region surrounding the carotid sheath. From the same patient, subcutaneous neck adipose tissue was obtained from the neck at the surgical incision. Tissue pairs were taken from $n = 45$ patients recruited at University Medical Center Ulm. We used $n = 26$ pairs for immunohistology ($n = 26$, 14 male, 12 female, age 56 ± 15 years, BMI 26.8 ± 4.4 kg m^{-2}, Table 1 and Appendix: Supplementary Table S2) and tissue mRNA samples from $n = 14$ patients ($n = 14$, 6 male, 8 female, age 58 ± 17 years, BMI 28.0 ± 6.7 kg m^{-2}) (Table 1). From the whole group of 45 patients, we had the chance to isolate progenitor cells from 12 patients ($n = 12$, 8 male, 4 female, age 48 ± 18 years, BMI 27.3 ± 5.3 kg m^{-2}, Table 2) which were taken into cell culture. Age, body mass index, and serum concentrations of triiodothyronine (T3) were recorded. The study was approved by the ethical committee of Ulm University and all patients gave written informed consent.

2.2. Histochemistry

Tissues were fixed in 4% paraformaldehyde overnight and washed in PBS. They were incubated in 70%, 80%, 90% and 100% ethanol and xylene for 2 h each and were then transferred to paraffin for embedding. Staining on paraffin sections was performed with rabbit anti-UCP-1 antibody (Abcam, Cambridge, UK) and sections were counterstained with hematoxylin using established histochemical methods. Brown adipose tissue was defined by the presence of multivacuolar adipocytes staining positive for UCP1. Adipocyte size was estimated by measuring the area of the cells in the histological samples, using BZ-9000 analyzer software (Keyence, Neu-Isenburg, Germany), analyzing at least 50 cells per sample. Blinded analyses were performed independently by two investigators (VS and DT).

2.3. Cell isolation and culture

Isolation of stromal-vascular cells was performed according to established protocols (Hauner et al., 2001). Briefly, adipose tissue biopsies (approximately 30–500 mg) were digested with 200 U/ml collagenase (Type I, Sigma-Aldrich) for 60 min at 37 °C and were filtered through a 100 μm cell strainer. After centrifugation (10 min, 550 *g*), pellets were resuspended and seeded into 25 cm^2 cell culture flasks. Cells were cultured in DMEM:F12 containing 10% fetal calf serum, panthotenate (17 μM), and biotin (33 μM) until reaching preconfluence.

Progenitor cells reached ~80% confluence after 7–10 days of cultivation and were either harvested for RNA isolation or were reseeded and subjected to adipogenic differentiation as described before (Hauner et al., 2001). Briefly, cells were differentiated using a serum and albumin free medium (DMEM:F12 containing 0.01 mg/ml

Table 2
Patient characteristics from subjects where progenitor cells were isolated from tissue biopsies.

Subject	Diagnosis	Age	Gender	BMI	Adipocytes UCP1 mRNA/HPRT relative to sc adipocytes	Gene array	OCR
1	Thyroid carcinoma	20	m	23.3	11.3	x	
2	Nodular goiter	51	m	27.1	7.6	x	
3	Thyroid carcinoma	56	w	22.2	2.6	x	
4	Nodular goiter	66	m	23.7	9.1	x	
12	Thyroid carcinoma	18	w	23.7	2.8	x	
13	Hyperthyreodism	44	w	30.0	0.2		
24	M. Basedow	23	w	28.4	5.1	x	
29	Nodular goiter	20	m	39.6	23.6		
42	Larynx carcinoma	67	m	34.9	5.1		x
43	Larynx carcinoma	68	m	25.4	5.8		x
44	Larynx carcinoma	52	m	24.8	30.5		
45	Oropahrynx carcinoma	56	m	23.7	3.9		x