Human SGBS Cells – a Unique Tool for Studies of Human Fat Cell Biology

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Summary

The human Simpson-Golabi-Behmel syndrome (SGBS) preadipocyte cell strain provides a unique and useful tool for studies of human adipocyte biology. The cells originate from an adipose tissue specimen of a patient with SGBS. They are neither transformed nor immortalized, and provide an almost unlimited source due to their ability to proliferate for up to 50 generations with retained capacity for adipogenic differentiation. So far, the cells have been used for a number of studies on adipose differentiation, adipocyte glucose uptake, lipolysis, apoptosis, regulation of expression of adipokines, and protein translocation. The cells are efficiently differentiated in the presence of PPARγ agonists and in the absence of serum and albumin. SGBS adipocytes respond to insulin stimulation by increasing glucose uptake several-fold (EC$_{50}$ approximately 100 pmol/l), and by very effectively inhibiting (IC$_{50}$ approximately 10 pmol/l) catecholamine-stimulated lipolysis.

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Introduction

Cell culture techniques offer the advantage that the effects of single factors and hormones can be studied under chemically defined conditions. This methodology is an optimal tool to elucidate mechanisms of e.g. cell development, hormone action, or metabolism. In vivo, the physiological function of a specific cell type or organ depends on its communication with the environment. As a component of a complex, highly integrated organism, adipose tissue depots interact and integrate substrate metabolism not only with surrounding but also with distant tissues. Thus, to fully understand and verify the physiological importance of any in vitro finding, it is essential to perform in vivo studies to account for cross-talk and substrate fluxes between various tissue.

The process of adipogenic differentiation as well as the metabolic and endocrine function of adipocytes has been primarily investigated in preadipocyte clonal cell lines of murine origin, which are all aneuploid. Furthermore, adipose precursor cells derived from the stromal-vascular fraction of adipose tissues from various species including humans have been used for in vitro studies in order to have a more physiological cell system. These cells are diploid but have a limited life span. An overview of cellular models of fat cell biology is given in table 1. In general, studies on human primary preadipocytes and adipocytes have been hampered by the availability of tissue, limited supply of cells, and to some extent the variability of adipose tissue from different donors. Few human cell lines are available (table 1), and some of them have been transformed or originate from adipose tissue tumors. In 2001, we introduced the human Simpson-Golabi-Behmel syndrome (SGBS) preadipocyte cell strain which is characterized by a retained capacity to adipogenic differentiation [1]. SGBS preadipocytes proliferate in vitro for many generations. Adipogenic differentiation is achieved under serum-free culture conditions by ex-

Table 1. In vitro models for studying adipocyte biology

Cell line	Species	Origin
Totipotent		
Embryonic stem cells	mouse	mouse blastocysts
Multipotent		
10T1/2	mouse	mouse embryo
3T3-F442A	mouse	Swiss mouse embryo (3T3 cells)
Unipotent		
Immortalized hMSC	human	
3T3-L1	mouse	Swiss mouse embryo
Ob17	mouse	epididymal fat pad of ob/ob mouse
TA1	mouse	subclone of 10T1/2 treated with 5-azacytidine
Lisa-2	human	liposarcoma
Cell strains		
Primary	rat	WAT from all depots of newborn and adult
Primary	mouse	WAT from all depots of newborn and adult
Primary	human	WAT from all depots of newborn and adult
Adult stem cells	mouse, rat, human	bone marrow
SGBS	human	Sc WAT of an infant with SGBS

hMSC = Human mesenchymal stem cell.

posure to a mixture of insulin, triiodothyronine, cortisol, and a PPARγ agonist. Even after proliferation for up to 50 generations, the cells retain their capacity for adipose differentiation as opposed to primary human preadipocytes which quickly loose their ability to differentiate when multiplied in vitro. SGBS cells have been secured and have now been multiplied into a cell bank for almost unlimited use. In the following sections, we describe the optimal differentiation procedure for human SBGS preadipocyte cells along with a summary of results of studies using human SGBS cells demonstrating how these cells have so far been used for studying adipocyte function.

Fig. 1. Optimal differentiation protocol for human SGBS cells. Differentiation is started (day 0) by incubating cells in a serum-free differentiation medium (2 μmol/l rosiglitazone, 25 nmol/l dexamethasone, 0.5 mmol/l methylisobuthylxantine, 0.1 μmol/l cortisol, 0.01 mg/ml transferrin, 0.2 nmol/l triiodotyronin, and 20 nmol/l human insulin). After 4 days, medium is changed, and cells are further cultured in medium supplemented with 0.1 μmol/l cortisol, 0.01 mg/ml transferrin, 0.2 nmol/l triiodotyronin, and 20 nmol/l human insulin.
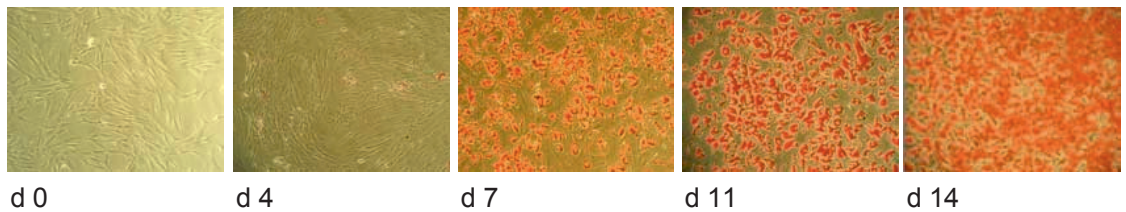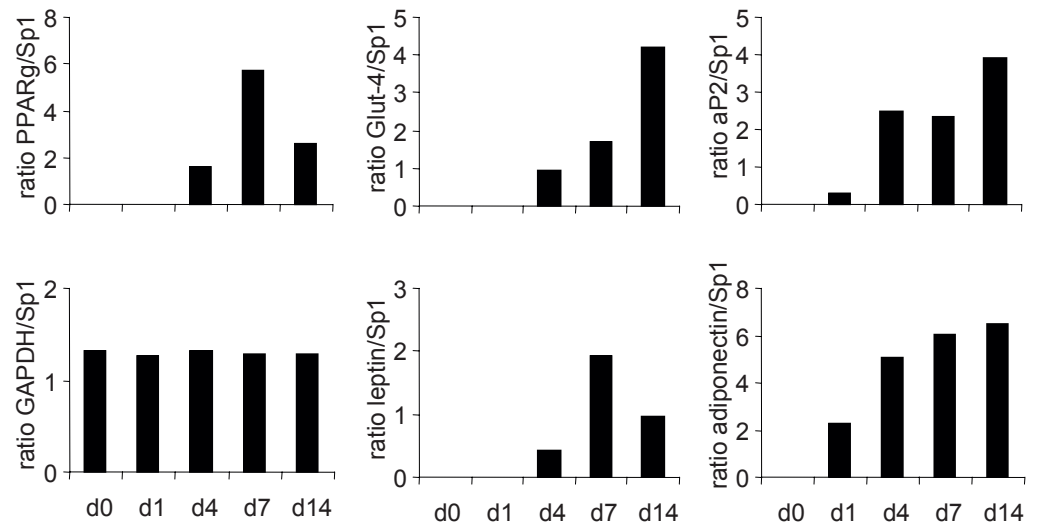
SGBS

SGBS preadipocytes have been named according to their origin. The cells were originally prepared from an adipose tissue specimen of a diseased patient with SGBS. SGBS is a complex, X-linked congenital overgrowth syndrome with features that include macroglossia, macrosomia, and renal and skeletal abnormalities as well as an increased risk of embryonal cancers [2]. The majority of cases of SGBS appear to arise as a result of either deletions or point mutations within the glypican-3 (GPC3) gene at Xq26, one member of a multigene family encoding for at least 6 distinct glycosylphophatidylinositol-linked cell surface heparan sulfate proteoglycans [2]. However, by sequencing the entire GPC3 gene in our cell strain, we were not able to detect any mutation in CPC3 (Felicity Newell, unpublished results) suggesting a mutation in another yet unknown responsible gene [2].

Adipogenic Differentiation of SGBS Cells

Over the past years, we have developed and optimized a protocol for SGBS fat cell differentiation which enables us to achieve an adipogenic differentiation rate of > 90% up to generation 50. The optimized differentiation scheme is depicted in figure 1. Typically, 5,000 cells/well at generation 30–60 (corresponding to passage 10–20) are grown in 12-well plates for 3 days to near confluence in DMEM/F12 containing 10% fetal calf serum (FCS). The procedure was successfully scaled down to 96- and 384-well plates. Differentiation is started (day 0) by washing cells 3 times with phosphate buffered saline (PBS) and then changing to a serum- and albumin-free differentia-

Fig. 2. Adipogenic differentiation of SGBS cells. **A** Microphotographs of SGBS cells during adipose differentiation cultured according to the differentiation protocol. Intracellular lipid droplets are clearly visible on culture day 7. On culture day 14, the cells are filled with high amounts of intracellular lipids. **B** Markers of adipocyte differentiation and function (mRNA expression of PPARγ, Glut-4, aP2, leptin, adiponectin) are induced and further up-regulated during the differentiation process. One representative experiment out of 3 performed is shown.

tion medium (DMEM/F12 supplemented with 2 μmol/l rosiglitazone, 25 nmol/l dexamethasone, 0.5 mmol/l methylisobuthylxantine, 0.1 μmol/l cortisol, 0.01 mg/ml transferrin, 0.2 nmol/l triiodotyronin, and 20 nmol/l human insulin). After 4 days, medium is changed, and cells are further cultured in DMEM/F12 supplemented with 0.1 μmol/l cortisol, 0.01 mg/ml transferrin, 0.2 nmol/l triiodotyronin, and 20 nmol/l human insulin. Within a few days, the cells start to accumulate lipids, and small lipid droplets are visible after approximately 1 week (fig. 2 A). Typical adipocyte marker genes such as aP2, Glut-4, leptin, and adiponectin are up-regulated during the adipogenic differentiation process (fig. 2 B). Additional information on primer sequences and polymerase chain reaction (PCR) conditions is given in [1]. After 14 days, > 90% of the cells are fully differentiated showing massive triglyceride accumulation. For functional studies involving insulin, cells are washed and deprived of insulin for at least 24 h. We want to point out that one of the major advantages of the SGBS model system is that adipogenic differentiation is performed in a chemically defined, serum- and albumin-free medium. In contrast, 3T3-L1 cells are differentiated in the presence of FCS.

Functional Characterization of Mature SGBS Adipocytes
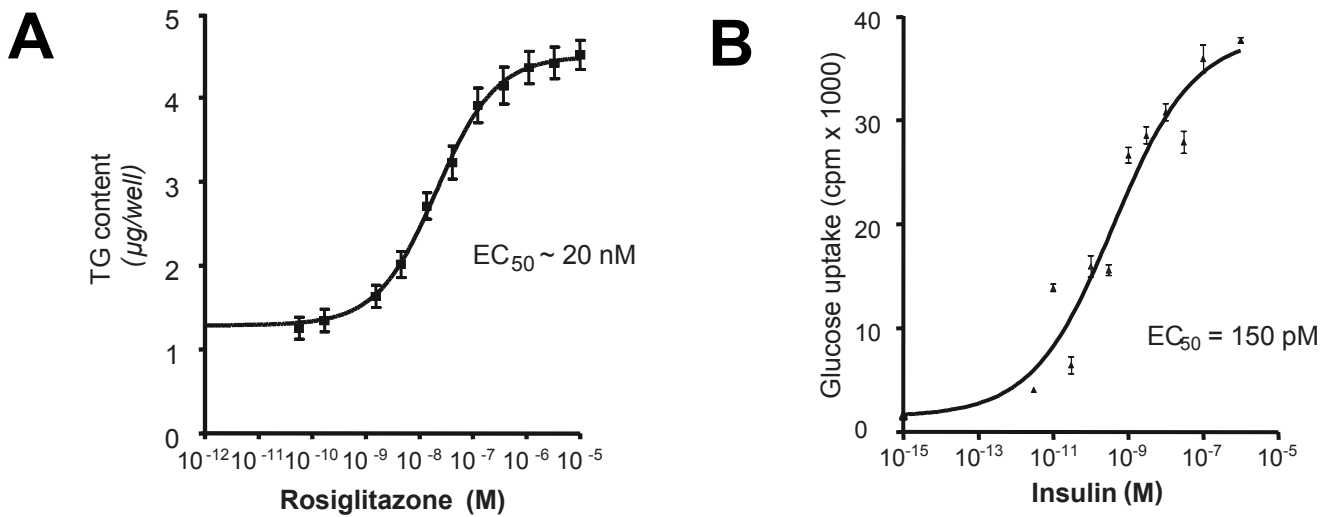
PPARγ-Dependent Differentiation

Establishing this cell strain, we initially found that the PPARγ agonist rosiglitazone as well as other PPARγ agonists markedly potentiate differentiation of SGBS preadipocytes [1]. Under optimal conditions, triglyceride accumulation at day 7 was increased 4-fold by rosiglitazone with an EC_{50} of approximately 20 nmol/l (fig. 3 A).

Insulin-Dependent Glucose Uptake

In fully differentiated SGBS adipocytes, following removal of insulin for 48 h, human insulin effectively increased U-^{14}C-glucose cellular uptake several-fold with an EC_{50} of approximately 70–110 pmol/l (fig. 3 B).

Isoproterenol-Stimulated Lipolysis and Anti-Lipolytic Effect of Insulin

The effects of isoproterenol with or without insulin in crossed dose-response curves are illustrated in figure 4. Both release of glycerol and fatty acids from the cells was measured following incubation for 4 h in the presence of 2% human serum albumin. Isoproterenol alone strongly induced

Fig. 3. PPARγ-dependent differentiation and insulin-stimulated glucose uptake. **A** Stimulation with rosiglitazone: intracellular accumulation of triglycerides. **B** Glucose uptake is increase several-fold upon insulin stimulation.
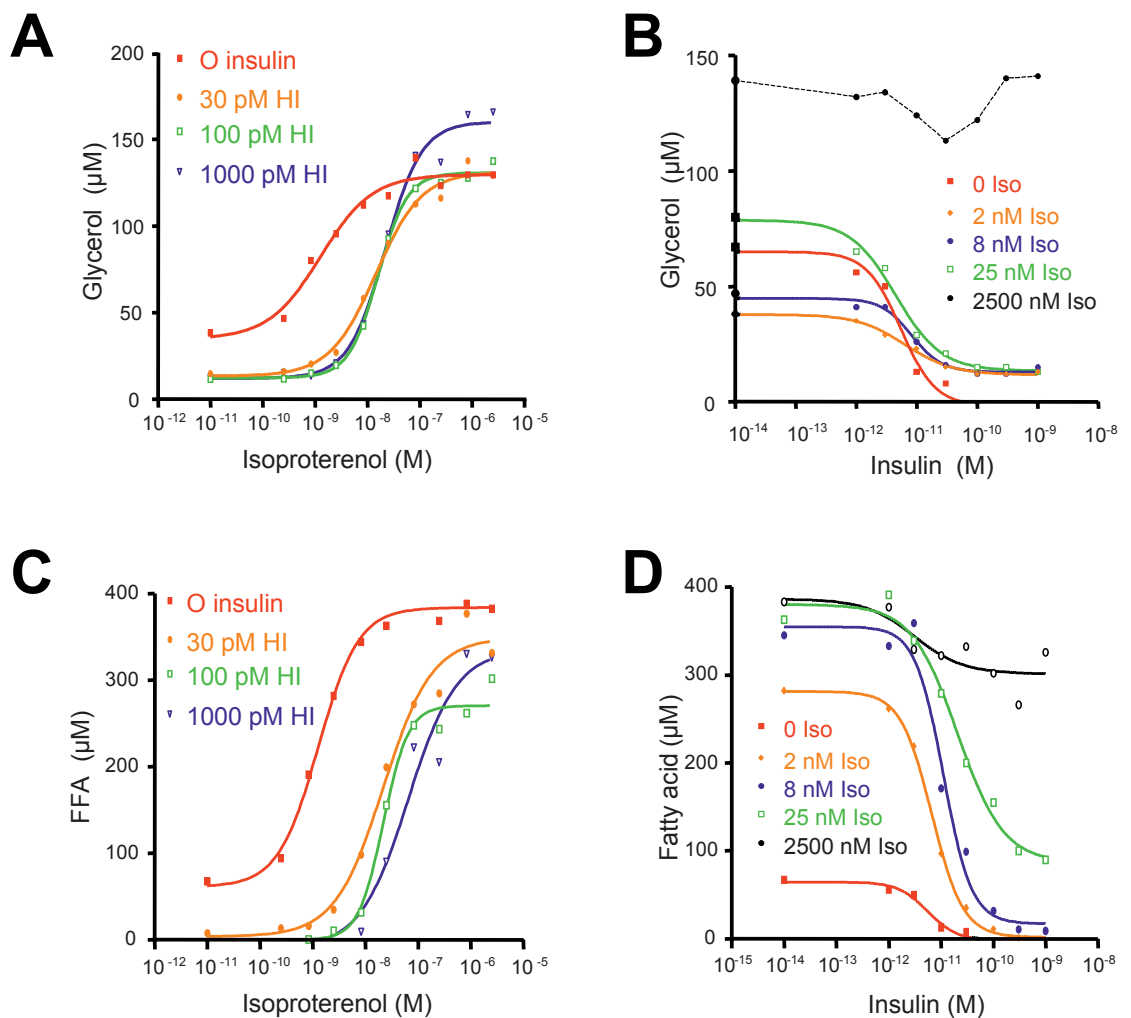
Fig. 4. Glycerol (**A, B**) and fatty acid mobilization (**C, D**) from SGBS adipocytes in response to different combinations of isoproterenol and insulin. (**A, C**) dose-response curves for isoproterenol at fixed insulin concentrations. (**B, D**) dose-response curves for the inhibitory effect of insulin at fixed isoproterenol concentrations.

Table 2. Studies published so far using SGBS cells

Year	Journal	Ref.
2001	Int J Obes	[1]
2003	Biochem Biophys Res Commun	[21]
2003	J Lipid Res	[7]
2004	Endocrinology	[18]
2004	J Biol Chem	[12]
2005	FEBS Lett	[22]
2005	Biochim Biophas Acta	[8]
2005	J Biol Chem	[23]
2005	Inflamm Res	[24]
2005	Prostaglandins Leukot Essent Fatty Acids	[24]
2005	Biochem J	[5]
2005	Biochem Biophys Res Commun	[19]
2005	Horm Metab Res	[13]
2006	J Clin Endocrinol Metab	[25]
2006	Diabetes	[26]
2006	J Clin Endocrinol Metab	[27]
2006	Obesity	[14]
2006	Am J Physiol Endocrinol Metab	[10]
2006	J Lipid Res	[11]
2006	Yonsei Med J	[17]
2006	FASEB J	[6]
2007	Horm Metab Res	[9]
2007	Plos Med	[29]
2007	Atherosclerosis	[15]
2007	J Clin Endocrinol Metab	[28]
2007	Cell Metab	[30]
2008	Breast Cancer Res Treat	[20]
2008	Arterioscler Thromb Vasc Biol	[31]

lipolysis, measured as release of glycerol and net fatty acid mobilization. Insulin was able to potently inhibit (IC_{50} approximately 10–20 pmol/l) submaximally stimulated lipolysis by isoproterenol (2, 8, or 25 nmol/l). These data are in accordance with those of primary rat adipocytes [3] showing again that maximal anti-lipolytic effects of insulin occur at submaximally stimulated lipolysis reflecting the changes in cAMP/protein kinase activity.

Some Study Data on Human Adipocyte Biology Using SGBS Cells

Since their first description in 2001, SGBS cells have been distributed to about 100 research laboratories all over the world, and several studies using this cell line as a model system have been published so far (table 2). Functionally, SGBS adipocytes behave like adipocytes differentiated in vitro from human preadipocytes obtained from the subcutaneous adipose tissue of donors. The cells also show a comparable gene expression pattern [1]. So far, most insights of the scientific literature into adipogenic differentiation were obtained using murine model systems [4]. SGBS cells are suited to study adipogenic differentiation and factors influencing this process in a human sys-

tem [5, 6]. Furthermore, these cells provide a unique model system to investigate the influence of food compounds or drugs on human fat cell function. For example, we and others have studied the effect of different isomers of conjugated linoleic acids (CLA) on adipocyte biology [7–9]. CLA have been shown to reduce fat mass in animal and cell culture models. However, controversial results were obtained in studies of supplementation of CLA in human subjects. Granlund et al. [7] showed that the trans10, cis12 CLA isomer prevents lipid accumulation in adipocytes by acting as a PPARγ modulator. This inhibition of lipid accumulation is associated with a tight regulatory cross-talk between early (PPARγ and C/EBPα) and late (LXRα, aP2, and CD36) adipogenic marker genes [8]. Our group showed that CLA promote apoptosis in SGBS adipocytes [9]. Other compounds studied in SGBS cells include PPARγ agonist pioglitazone [10, 11], PPARα agonist fenofibrate [12], human immunodeficiency virus (HIV) protease inhibitors ritonavir and atazanavir [13, 14], and HMG-CoA reductase inhibitor rosuvastatin [15].

In the last decade, it became more and more clear that adipose tissue is more than an organ storing energy in the form of triglycerides. It is now well recognized as an endocrine organ secreting more than 100 different factors [16]. Depending on the state of adipogenic differentiation, SGBS cells secrete a plethora of adipokines such as interleukins [13, 14, 17], insulin-like growth factor-1 (IGF-1) [18], plasminogen activator inhibitor-1 [15], leptin, and adiponectin [10, 13, 19]. Using SGBS adipocytes as model system, we studied the regulation of adiponectin expression [19]. Adiponectin was not expressed by human preadipocytes. Differentiation into adipocytes was necessary to induce an increasing expression of adiponectin in parallel to an increasing expression of adipocyte differentiation markers. Adiponectin protein synthesis and secretion occurred specifically in mature adipocytes, and may thus serve as a distinctive marker of adipocyte differentiation. Addition of serum during the course of differentiation as well as acutely to mature adipocytes significantly and concentration-dependently suppressed adiponectin to almost non-detectable levels, suggesting a strong humoral serum component of adiponectin down-regulation. This serum component is present in both obese and lean individuals with a tendency to a stronger effect in obese men and women. Withdrawal of adipogenic ingredients from the culture medium also resulted in a decrease of adiponectin expression and secretion, respectively. We identified insulin as a critical component to maintain adiponectin expression. Furthermore, pioglitazone was shown to increase secretion of high-molecular-weight adiponectin from adipocytes [10]. Ritonavir, a HIV protease inhibitor, reduced adiponectin expression [13, 14].

Interestingly, an Australian group has recently shown that aromatase, a member of the cytochrome P450 superfamily of enzymes which catalyses the rate-limiting step in the biosynthesis of estrogens, is expressed in SGBS preadipocytes [20]. Its expression and activity are strongly stimulated by forskolin

(FSK) and phorbol 12-myristate-13-acetate (PMA) treatment. These findings are comparable to those obtained in isolated primary human preadipocytes [20], and demonstrate that SGBS cells are a valuable model to study this specific endocrine function of preadipocytes and adipocytes.

Conclusion

The primary human SGBS preadipocyte cell strain offers an almost unlimited source of homogeneous human preadipocytes with high differentiation capacity. The cells have not been transformed or immortalized. They are easily cultivated in 96/384-well plates with high reproducibility, and are differentiated in the absence of any added serum or albumin. Once differentiated, the cells function and behave as mature adipocytes and show optimal responses in key functional assays. The exact molecular alteration behind the increased capacity to proliferate with retained ability to differentiate is at present not known. In conclusion, the human SGBS preadipocyte cell strain offers an excellent new tool for studies of human adipocyte biology.

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