In Depth Quantitative Proteomic and Transcriptomic Characterization of Human Adipocyte Differentiation using the SGBS Cell Line

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Most information on molecular processes accompanying and driving adipocyte differentiation are derived from rodent models. Here, a comprehensive analysis of combined transcriptomic and proteomic alterations during adipocyte differentiation in Simpson–Golabi–Behmel Syndrome (SGBS) cells is provided. The SGBS cells are a well-established and the most widely applied cell model to study human adipocyte differentiation and cell biology. However, the molecular alterations during human adipocyte differentiation in SGBS cells have not yet been described in a combined analysis of proteome and transcriptome. Here a global proteomic and transcriptomic dataset comprising relative quantification of a total of 14372 mRNA transcripts and 2641 intracellular and secreted proteins is presented. 1153 proteins and 313 genes are determined as differentially expressed between preadipocytes and the fully differentiated cells including adiponectin, lipoprotein lipase, fatty acid binding protein 4, fatty acid synthase, stearoyl-CoA desaturase, and apolipoprotein E and many other proteins from the fatty acid synthesis, amino acid synthesis as well as glucose and lipid metabolic pathways. Preadipocyte markers, such as latent, GATA6, and CXCL6, are found to be significantly downregulated at the protein and transcript level. This multi-omics data set provides a deep molecular profile of adipogenesis and will support future studies to understand adipocyte function.

Obesity and associated comorbidities, for example, diabetes, hypertension and cardiovascular diseases, represent the most common health risks worldwide and the prevalence has more than doubled in the last 35 years and already emerged in childhood.[1,2] Biologically, for the fat mass to accumulate besides proliferation, differentiation of fibroblast-like precursors cells (preadipocytes) into lipid-filled mature adipocytes is a major process of tissue expansion.[3] Hence, the understanding of the molecular events in this process of adipocyte development, differentiation, and biology is of growing importance.[4]

Preadipocyte cell lines, such as murine 3T3-L1, or primary adipocytes from mice, rats or humans serve as experimental models for human adipogenesis and are widely used to characterize adipogenic regulators and thereby provide substantial insights into the molecular processes regulating adipogenesis.[3] Nevertheless, there are some constraints on the generalizability to the physiology of human adipogenesis, as for example, 3T3-L1 cells

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are spontaneously immortalized aneuploid embryonic fibroblasts and they are derived from mouse. On the other hand, primary human preadipocytes cannot be expanded to high cell numbers without losing their capacity to differentiate into mature adipocytes.\[6\] Furthermore, there is high degree of interindividual variability for transient cultures from cells of individuals, hence limiting generalizability.

In 2001, a human preadipocyte cell line derived from a boy with the Simpson–Golabi–Behmel syndrome (SGBS) was established, which was shown to be a suitable model system for in vitro studies in adipocyte research.\[7–9\] SGBS preadipocytes are of human origin, diploid, differentiate in a serum free, chemically standardized medium, and allow for reproducible experiments as the cell numbers can be largely increased while the cells retain their good differentiation capacity for more than 30 generations.\[6,9\] The SGBS model has been used for numerous studies, such as pharmacological testing,\[10\] genetic influences on adipocyte biology,\[11,12\] and adipogenesis or the characterization of adipokines.\[13,14\] As proof of principle, the expression profiles of genes like GLUT4, leptin, or lipoprotein lipase (LPL) in differentiating SGBS preadipocytes have been shown to resemble those for primary human preadipocytes from adipose tissue biopsies.\[9\] A qualitative comparison of proteins secreted from SGBS preadipocytes and differentiated SGBS adipocytes has, however, not yet been performed.

Here we performed a comprehensive analysis on the protein and expression profile of SGBS cells during the time course of adipocyte differentiation. In order to characterize adipocyte differentiation on the proteome and transcriptome level, human SGBS preadipocytes cells were differentiated as described previously.\[8,9\] Briefly, subconfluent SGBS preadipocytes were differentiated in DMEM/Ham’s F12 containing 33 µM biotin, 17 µM pantothenate, 10 µg/mL apo-transferrin, 20 nm insulin, 10 nm hydrocortisone, and 0.2 nm triiodothyroxine for 10 days. For the first 4 days of differentiation the medium was additionally supplemented with 25 nM dexamethasone, 500 µM isobutylmethylxanthine, and 2 µM rosiglitazone (for detailed information see Supporting Information).\[7,13\]

For the proteome analysis, we used Stable Isotope Labeling in Cell Culture (SILAC) combined with LC-MS/MS for an accurate in-depth quantitative proteome analysis of intracellular and secreted proteins as described previously.\[15,16\] All experiments were performed in biological triplicates.

We identified 3737 intracellular proteins of which 2602 were reliably quantified in at least two of the three biological replicates. In secretome analysis, 390 proteins were identified of which 39 proteins included established adipogenic regulators and modulators such as apolipoprotein E (APOE) and adiponectin (ADIPOQ) were quantified. Overall, almost half of the intracellular and extracellular proteins were differentially expressed during adipocyte differentiation (Figure 1A).

The analysis of the subcellular localization of proteins according to the GO (gene ontology) database annotation showed enrichment of cytoplasmic proteins, but also of nuclear and
1135 intracellular proteins and 18 secreted proteins were determined to be significantly regulated with a log2 fold change of >0.5 and a p-value of <0.01 (two tailed t-test with unequal variance) (Figure 1C, Table S1, Supporting Information).

All regulated proteins were clustered according to the annotated GO biological processes and canonical pathways as well as to their assignment to metabolic pathways as provided by the Kyoto encyclopedia of genes and genomes (KEGG). The majority of the regulated proteins are involved in metabolic pathways, for example, tricarboxylic cycle and mitochondrial respiratory chain, as well as fatty acid beta-oxidation (Figure 2A). Analysis of molecular protein functions showed an enrichment of poly(A) RNA binding proteins, cadherin-based cell-cell interaction proteins, oxidoreductases, ribosomal proteins, and actin binding proteins (Figure 2B). KEGG pathway analysis of the differentially expressed proteins revealed that amino acid metabolism, cell respiration, and fatty acid metabolism were among the most significantly regulated (Figure 2C). Several proteins involved in the peroxisome proliferator-activated receptor (PPARγ) signaling pathway were upregulated, as described earlier.\(^\text{[17,18]}\) Taken together, differentiation of adipocytes is accompanied by a large degree of differential protein expression with lipid and amino acid metabolism related pathways being the most affected ones.

For a comprehensive analysis of the proteome in relation to the transcriptome, we further analyzed the gene expression profile during adipogenesis by an Affymetrix GeneChip Expression Analysis. For this, 5 µg of total RNA were processed for microarray analysis. Reverse transcription, in vitro transcription, fragmentation, and hybridization of the samples were performed according to the manufacturer’s recommendations. Samples were hybridized to HG U133A 2.0 arrays. Scanning and first data processing were executed with GeneChip operating software. The sample cell intensity (CEL) files were imported to GeneSpring GX 7.3. (Agilent, Palo Alto, USA) using the RMA algorithm for raw data processing.\(^\text{[19]}\) Each gene on every array was normalized to the median of all measurements of that gene.
A gene was generally considered as not expressed when the raw expression signal was below 100 in all samples. Reassuringly, in principal component analysis, samples are grouped into distinct stages of differentiation (Figure S1, Supporting Information). For statistical analysis samples were grouped into preadipocytes \((n = 3)\), early differentiation \((n = 3)\), intermediate phenotype \((n = 2)\), and mature adipocytes \((n = 3)\) according to expression profiles (Supporting Information). The variances estimated by the Cross-Gene Error Model were further used for statistical testing. Data were deposited in Proteomics identifications database (PRIDE) (ID PXD012476) and GEO data sets GSE123385.

In total, 14372 out of 22277 tested transcripts were expressed in SGBS cells at any time point of differentiation, encoding for 9346 unique proteins. 1520 of those were quantified at protein level as well (Table S2, Supporting Information). 313 genes (3.3%) represented by 404 (2.8%) transcripts had expression changes >fivefold during adipocyte differentiation (Table S3, Supporting Information). Correlation analyses revealed that regulation at the protein level highly corresponded to the transcriptome level comparing adipocytes to the preadipocytes (Figure S2, Supporting Information).

Next, we analyzed typical adipocyte marker expression during adipogenesis at the transcriptome and proteome level for testing the robustness of the molecular signature of differentiation. We found an upregulation of proteins involved in adiponectin expression, cholesterol synthesis (acyetyl-CoA acetyltransferase, lipase E), amino acid metabolism (aminocylase), and of factors involved in lipogenesis and lipolysis (acyetyl-CoA carboxylase \(\alpha\) and \(\beta\) (ACACA, ACACB)). Additionally, typical markers of mature adipocytes like \(LPL\), fatty acid binding protein 4 (\(FABP4\)), fatty acid synthase (\(FASN\)), stearoyl-CoA desaturase and \(APOE\) were upregulated. In contrast, latexin (\(LXN\)), an inhibitor of carboxypeptidase A1, 2 and 4 were observed to be downregulated. We identified the three preadipocyte markers, \(LXN\), transcription factor GATA-6 (\(GATA6\)), and C-X-C motif chemokine 6 (\(CXCL6\)), and the four mature adipocyte markers \(LPL\), ACACB, APOE, and the ATP citrate lyase (\(ACLY\)) (Figure S3, Supporting Information).

In order to validate the marker gene expression profiles and to identify those with the highest adipocyte status prediction ability, we performed TaqMan RT-PCR in three independent SGBS differentiation experiments (Supporting Information). In SGBS cells, we were able to validate the gene expression profiles of all seven marker genes and showed a significant regulation during SGBS differentiation. Five marker genes validated with qPCR in SGBS cells were also regulated in the same direction at the proteome level (Figure 3). ACACB, ACYL, ADIPOQ, and APOE were significantly upregulated in mature adipocytes, whereas \(LXN\) was significantly downregulated.

Taken together, SGBS adipocytes exhibit key features and key protein and mRNA signatures of primary mammalian adipocytes, for example, morphological characteristics as well as molecular hallmarks like the activation of the PPAR\(\gamma\) pathway. Here, we show that also the expression of several typical adipokines (e.g., \(ADIPOQ\)) and mature adipocyte markers (e.g., \(LPL\), \(FABP4\), \(FASN\)) are induced in SGBS cell differentiation. Moreover, the obtained data reveal a metabolic programming during adipogenesis, with lipid metabolism related pathways being the most affected.

We expect the proteomics and transcriptomics data presented here to be of interest for the further application and establishment of SGBS cells as a well-characterized model system for adipocyte differentiation as well as for the identification of marker genes, which can be used to characterize human fat tissue biopsies in terms of composition and differentiation state. Our data may facilitate and guide future studies focusing on the regulation of adipogenesis and human adipocyte signatures.

**Figure 3.** Validation of marker genes during adipogenesis. A) LC-MS/MS analysis: Bar charts show Log2 fold change for selected proteins after differentiation into mature adipocytes (day 10). Significantly differences to the control (day 0) are highlighted \((^*p\text{-value} \leq 0.05, ^{**p\text{-value}} \leq 0.01)\). B) RT-qPCR analysis: Bar charts show Log2 fold change for selected marker genes after differentiation into mature adipocytes. Significantly differences are displayed relative to the control (day 0) are highlighted \((^{**p\text{-value}} \leq 0.01, ^{****p\text{-value}} \leq 0.0001)\).
Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

M.B., M.W., W.K., A.K., and M.v.B. initiated the study. A.K. and S.K. supervised the study. S.K., K.L., K.S., L.K., and P.B. drafted the manuscript and analyzed the data. C.K. and S.K. conducted the mass spectrometric analysis and preparation of the protein samples; D.F. and P.B. performed wet lab work with the cells; S.B. and S.G. performed the metabolite analysis; W.O. performed the statistical analysis. S.K., K.L., M.v.B., A.K., K.S., L.K., S.B., P.B., and M.W. discussed and interpreted the data. All authors revised the draft manuscript and read and approved the final manuscript.

Keywords

adipocyte differentiation, adipogenesis, obesity, proteomics, Simpson–Golabi–Behmel syndrome cells

[1] M. Geserick, M. Vogel, R. Gausche, T. Lipek, U. Spielau, E. Keller, R. Pfaffle, W. Kiess, A. Körner, N. Engl. J. Med. 2018, 379, 1303.
[2] NCD Risk Factor Collaboration, Lancet 2016, 387, 10026.
[3] K. Landgraf, D. Rockstroh, I. V. Wagner, S. Weise, R. Tauscher, J. T. Schwartzze, D. Löffler, U. Buhligen, M. Wabitsch, W. Kiess, M. Bluher, A. Körner, Diabetes 2015, 64, 1249.
[4] A. Must, J. Spadano, E. H. Coakley, A. E. Field, G. Colditz, W. H. Dietz, JAMA. J. Am. Med. Assoc. 1999, 282, 1523.
[5] H. Sadie-Van Gijsen, J. Cell. Physiol. 2019, 234, 2399.
[6] F. J. Ruiz-Ojeda, A. I. Ruperez, C. Gomez-Llorente, A. Gil, C. M. Aguillera, Int. J. Mol. Sci. 2016, 1040, 17.
[7] E. H. Allott, E. Oliver, J. Lysaght, S. G. Gray, J. V. Reynolds, H. M. Roche, C. P. Pidgeon, Clin. Transl. Oncol. 2012, 14, 774.
[8] P. Fischer-Posovsky, F. S. Newell, M. Wabitsch, H. E. Tornqvist, Obes. Facts 2008, 1, 184.
[9] M. Wabitsch, R. E. Brenner, I. Melzner, M. Braun, P. Moller, E. Heinze, K. M. Debatin, H. Hauner, Int. J. Obes. 2001, 25, 8.
[10] D. Löffler, K. Landgraf, A. Körner, J. Kratzsch, K. C. Kirkby, H. Himmerich, J. Psych. Res. 2016, 72, 37.
[11] F. Bernhard, K. Landgraf, N. Kloting, A. Berthold, P. Buttnner, D. Friebe, W. Kiess, P. Kovacs, M. Bluher, A. Körner, Diabetologia 2013, 56, 311.
[12] D. Rockstroh, D. Löffler, W. Kiess, K. Landgraf, A. Körner, Adipocyte 2016, 5, 283.
[13] A. Körner, M. Wabitsch, B. Seidel, P. Fischer-Posovsky, A. Berthold, M. Stumvoll, M. Bluher, J. Kratzsch, W. Kiess, Biochem. Biophys. Res. Commun. 2005, 337, 540.
[14] D. Löffler, K. Landgraf, D. Rockstroh, J. T. Schwartzze, H. Dunzendorfer, W. Kiess, A. Körner, Int. J. Obes. 2017, 41, 112.
[15] C. Goettsch, S. Kliemt, K. Sinningen, M. von Bergen, L. C. Hofbauer, S. Kalkhof, J. Mol. Cell. Cardiol. 2012, 53, 829.
[16] J. R. Schmidt, S. Vogel, S. Moeller, S. Kalkhof, K. Schubert, M. von Bergen, U. Hempel, J. Cell Biochem. 2018, 120, 5.
[17] Y. Barak, M. C. Nelson, E. S. Ong, Y. Z. Jones, P. Ruiz-Lozano, K. R. Chien, A. Koder, R. M. Evans, Mol. Cells 1999, 4, 585.
[18] E. D. Rosen, P. Sarraf, A. E. Troy, G. Bradwin, K. Moore, D. S. Millstone, B. M. Spiegelman, R. M. Mortensen, Mol. Cell 1999, 4, 611.
[19] B. Harr, C. Schlotterer, Nucleic Acids Res. 2006, 34, e8.
[20] C. R. Yeo, M. Agrawal, S. Hoon, A. Shabbir, M. K. Shrivastava, S. Huang, C. M. Khoo, V. Chhay, M. S. Yassin, E. S. Tai, A. Vidal-Puig, Sci. Rep. 2017, 7, 4031.