SMOX and SMS Responsible for Polyamine Metabolism Enhanced Adipogenesis

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Abstract

Adipose tissue regulating carbohydrate and lipid metabolism had been extensively focused. However, the regulation of amino acid metabolism during adipocyte differentiation remained detailed. Here we applied RNA-Seq technique to establish the transcriptional landscapes of amino acid metabolism during adipogenesis. We totally screened 17 differentially expressed genes (DEGs) for amino acid metabolism at 7, 14, 21, and 28 days during adipogenesis from human mesenchymal stem cells (hMSCs), especially with 13 up-regulated genes most prevalent in our adipogenic anecdote. Small molecule metabolic process was the most enriched biological process following by oxidation-reduction process. Interestingly, the enforced expression of SMOX (spermine oxidase) responsible for polyamine metabolism in arginine and proline metabolism pathway facilitated adipogenesis more than SMS (spermine synthase) using RNA interference (RNAi). The established potential regulatory network further suggested that adipocyte differentiation tightly related with the basal metabolism of amino acid metabolism with the partially confirmed SMS-SMOX-PPARG signaling pathway. It would highlight the effect of adipogenesis on amino acid metabolism in adipocyte biology and provide the potential treatment strategy for the study of fat metabolic diseases.

Introduction

As we known, the consumption of unutilized calories induced a metabolic state promoting the commitment of human mesenchymal stem cells (hMSCs) to become preadipocytes, followed by their differentiation into mature adipocytes known as the primary site for fat storage [1–3]. It had been clinically well established for the dysfunction of the endocrine and paracrine signaling usually responsible for the important regulatory functions of adipocytes leading to metabolic disorders and diseases, such as obesity, type 2 diabetes mellitus (T2DM), inflammation, and insulin resistance [4–8]. As a highly orchestrated process, adipocyte differentiation regulated glucose and lipid homeostasis by storing the excess nutrients in lipid droplets and releasing the bioenergetic substrates via lipolysis [9–10].

Recently, the studies involving in the relationship between amino acid and adipogenic differentiation attracted an increasing focus. Amino acids had been confirmed as the metabolic switch fueling adipogenic differentiation, such as branched-chain amino acid (BCAA) [11], homocysteine [12], arginine [13], and polyamine [14]. Especially, it had well documented that the inhibition of BCAA catabolism thoroughly compromised adipogenesis [11]. Hydroxyproline, proline, lysine, glycine, and alanine also showed a potential to induce adipogenic effects in retinal pericytes [15]. However, relatively little is uncovered for the whole profiling of amino acid metabolism during adipogenesis.

In our previous study, we established the whole transcriptional profiling of cellular metabolism during adipogenesis [16]. Here, we further concentrated our attention on the transcriptional landscapes of amino acid metabolism during adipogenesis using RNA-Seq technique. Among 17 DEGs for amino acid metabolism at 7, 14, 21, and 28 days, we confirmed that the enforced expression of SMOX (spermine oxidase) responsible for polyamine metabolism in arginine and proline metabolism pathway facilitated

adipogenesis more than SMS (spermine synthase) using RNA interference (RNAi) by lentiviruses system. The established potential regulatory network further indicated that adipocyte differentiation tightly related with the basal metabolism of amino acid metabolism with the partially confirmed SMS-SMOX-PPARG signaling pathway. This study would highlight the effect of adipogenesis on amino acid metabolism in adipocyte biology and provide the potential treatment strategy for the study of fat metabolic diseases.

Material And Methods

hMSCs isolation and culture

Identified in our previous study [16], the hMSCs were isolated following the protocols with slight modification [17–18]. Adipogenic differentiation assays were developed following the method with the slight modifications [19–20]. In an Model 3100 series Forma Series $\square$ Water Jacket CO₂ incubator (Thermo Fisher Scientific, Ohio, United States), we cultured the hMSCs in 5.0 mL hMSCs Basal Medium (Cyagen bioscience, Inc., Santa Clara, CA, USA) supplemented with FBS (fetal bovine serum), penicillin-streptomycin, and L-glutamine in 25 cm² flasks (Corning Incorporated, Corning, New York, USA) at 37°C with 5% CO₂ and 95% humidity.

Human adipocyte differentiation

Expanded to passage 6, the hMSCs with 80–90% of the final confluence were stimulated with adipogenic cocktails of hMSCs Basal Medium including 1.0 μ M dexamethasone, 0.01 mg/mL insulin, and 0.5 mM 3-isobutyl-1-methyl-xanthine (Sigma, St. Louis, Mo, USA) on day 0. Adipogenic potential of hMSCs was assessed following the documented method with slight modification [21–22]. Briefly, the hMSCs were fixed with 3.7% formaldehyde for 30 min at room temperature after washed with phosphate buffered saline (PBS) (pH 8.0) buffer twice. And then, the hMSCs were stained with Oil Red O (Cyagen bioscience, Inc., Santa Clara, CA, USA) for 1 h after washing three times with PBS buffer. Cell morphology and the formation of lipid droplets of adipocytes were photographed using a fluorescence microscope (IX73, Olympus, Tokyo, Japan).

RNA isolation and RNA-Seq sequencing and analysis

Following the manufacturer's instructions, the total RNA isolated from the adipogenic samples using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) was stored in liquid nitrogen for the further analysis. The freshly isolated RNA samples were for RNA sequencing after purified using the NucleoSpin RNA clean-up kit (Macherey-Nagel, Düren, Germany) and qualified by Bioanalyzer 2200 (Aligent Technologies, Santa Clara, CA, USA).

In our previous study [23], RNA-Seq sequencing was developed by NovelBio Bio-Pharm Technology Co., Ltd, Shanghai, China. Additional remarks, the Ion Total RNA-Seq kit v2.0 (Life Technologies, Santa Clara,

CA, USA) following the manufacturer’s instructions was used to prepare RNA of RIN (RNA integrity number) > 8.0 to construct the complementary DNA (cDNA) libraries for single-end sequencing of proton-sequencing process.

Here, we systematically established the transcriptional landscapes of amino acid metabolism during adipogenesis depending on KEGG pathway. We defined an absolute value of $\log_2$ ratio ≥ 2.0 as the threshold of the differentially expressed genes (DEGs), and the significant DEGs simultaneously met a false discovery rate (FDR) < 0.001 and an absolute value of $\log_2$ ratio ≥ 2.0 . We set the cutoff of p -value < 0.05 as the differential expressed biological process for GO (Gene Ontology) analysis.

qRT-PCR assays

Here, the relative gene expression level was determined using qRT-PCR (quantitative real-time PCR). 2.0 μ g RNA was used to synthesize cDNA using Oligo (dT₁₈) primer complementary base pairing with poly (A) mRNA using the cDNA synthesis kit (Torobo Co., Osaka, Japan) on a 7500 Real Time PCR System (ABI, Foster, CA, USA) from Bio-Rad Laboratories (Hercules, CA, USA). A SYBR Green Realtime PCR Master Mix (Torobo, Osaka, Japan) was used as a fluorescent dye, and amplifications were performed according the manufacturer’s guidelines. The amplification program was as follows: 94 °C for 5 min for predenaturation, and then 35 cycles at 94°C for 1 min for denaturation, 52°C for 30 sec for annealing, and 72 °C for 30 sec for extension. The oligonucleotide primers synthesized by Generay Biotech Co., Ltd (Shanghai, China) were listed in Table 1. ACTB (actin beta) was used as the internal control [24]. All experiments were carried out in triplicates.

Table 1
Oligonucleotide primers used in this study.

Gene	Product	Forward primer (5'-3')	Reverse primer (5'-3')
ACTB	Actin beta	CGAGGACTTTGATTGCACATTG	AGAGAAGTGGGGTGGCTTTTAG
CEBPG	CCAAT enhancer binding protein gamma	AGCTTCGGGAATTGTGAG	AACTATGCTGGACCTGCC
FASN	Fatty acid synthase	AGAGCTACATCATCGCTG	AGAAGTCAACACGAGCTT
LPL	Lipoprotein lipase	GGGAACCCGACTGTGAAA	CGACTGTTTTCCCTTCCG
PPARG	Peroxisome proliferator activated receptor gamma	TGCAAGGGTTTCTTCCGG	ATCCCCACTGCAAGGCAT
SMOX	Spermine oxidase	TAACTCGTGACCTCCAGC	GCGGCTAGCTCTACAGAA
SMS	Spermine synthase	CACAGCGAAGACTGCTAAATGC	TCCCTCCAGCAAAACAACGATG

Validation studies of the candidate genes

In order to investigate gene function of our screened target genes, we strengthened the expression of SMS and SMOX differentially down-regulated during adipogenesis with small interfering RNA (siRNA) using a lentivector expression system. Lentiviral vector pGV492 digested with *Bam*HI/*Age*I was used to overexpress SMS (NM_004595) and SMOX (NM_175839). The primer sequences of SMS were as follows: 5'-AGGTCGACTCTAGAGGATCCCGCCACCATGGCAGCAGCACGGCACAGCACG-3' and 5'-TCCTTG TAGTCCATACCGGGTTTAGCTTTCTTCCAAACAG-3', and those of SMOX were as follows: 5'-AGGTCGACTCTAGAGGATCCCGCCACCATGCAAAGTTGTGAATCCAGTGG-3' and 5'-TCCTTG TAGTCCATACCGGTCCCCTGCTGGAAGAGGTCTCG-3'. All the lentiviruses, including SMS-siRNA, SMOX-siRNA, and the negative control, were prepared by GeneChem Co., LTD (Shanghai, China).

After expanded to passage 6 approximately with 20% confluence, the hMSCs were separately transfected with SMS-siRNA and SMOX-siRNA lentiviruses using a multiplicity of infection (MOI) of 10 and HitransG Transfection Reagent P (Genechem Inc., Shanghai, China). Transfection efficiency was confirmed according to fluorescence intensity of GFP (Green fluorescent protein) expression under a fluorescence microscope (IX73, Olympus, Tokyo, Japan).

Results

Adipogenic differentiation

Here, we assessed adipogenic potential of hMSCs via Oil Red O staining assays (Fig. 1). No lipid droplet appeared at 0 day, and the most obvious phenotypic changes was at 7 days after hMSCs differentiating to adipocytes with lipid-droplets accumulating (Fig. 1a; 1b). Specially, the expansion of size and number of lipid droplets increased from 7 to 28 days (Fig. 1b-f). It indicated our hMSCs differentiating along adipogenesis.

Transcriptional landscapes of amino acid metabolism during adipogenesis

Here, RNA-Seq technique was performed to establish the transcriptional landscapes of amino acid metabolism during adipogenesis. 17 DEGs were identified during adipogenesis (Fig. 2). Furthermore, 13 up-regulated genes were most prevalent at four sampling points in our adipogenic anecdote, and thus suggested adipogenic differentiation facilitated the gene expression of amino acid metabolism pathway. As amino acid metabolism was a high energy demanding process, the facilitation of amino acid metabolism at transcriptional level would be the supply process of the essential energy.

Nine DEGs (MAOA, ALDH1A3, AOC2, AOC3, SMS, ALDH4A1, ASS1, GLUL, and SMOX) got involved in more metabolic pathways of amino acid metabolism, including arginine and proline metabolism, glycine,

serine and threonine metabolism, histidine metabolism, phenylalanine metabolism, tryptophan metabolism, tyrosine metabolism, cysteine and methionine metabolism, aspartate and glutamate metabolism, and arginine biosynthesis. For the DEG of MAOA (monoamine oxidase A), it related with six amino acid metabolism pathways, such as arginine and proline metabolism, glycine, serine and threonine metabolism, histidine metabolism, phenylalanine metabolism, tryptophan metabolism, and tyrosine metabolism. Seven DEGs (ALDH1A3, AOC2, AOC3, SMS, GGT1, GGT5, and SMOX) were the most abundant for the other amino acids metabolism, including beta-alanine metabolism, glutathione metabolism, taurine and hypotaurine metabolism, and beta-alanine metabolism was the most prevalent. Above all, the gene transcriptional landscapes of amino acid metabolism were strong in response to adipogenesis.

The above DEGs were also matched along with biological process by GO analysis (Fig. 3). Based on one gene corresponding to multiple GO, we identified the differential expressed biological processes ($p_value < 0.05$), such as MAOA (GO:0044281 and GO:0055114), ALDH1A3 (GO:0043065 and GO:0055114), AOC2 (GO:0055114), AOC3 (GO:0006954, GO:0007155, and GO:0055114), SMS (GO:0044281), ALDH4A1 (GO:0044281), ASS1 (GO:0044281), GGT1 (GO:0044281), GGT5 (GO:0006954 and GO:0044281), GLUL (GO:0044281 and GO:0050679), SMOX (GO:0044281 and GO:0055114), ADH1A (GO:0044281, GO:0055114), ADH1B (GO:0044281, and GO:0055114), ADH1C (GO:0044281 and GO:0055114), CYP1B1 (GO:0001525, GO:0008202, GO:0043542, GO:0044281, GO:0055114), and GPX (GO:0055114). In all, small molecule metabolic process (GO:0044281) was the most enriched biological process following by oxidation-reduction process (GO:0055114). As we known, all amino acids derived from the intermediates in glycolysis, the tricarboxylic acid cycle (TCA), or pentose phosphate pathway (PPP). The two differential biological processes would be the key way for the energy supply.

SMS and SMOX enhanced adipogenesis

The two genes SMS and SMOX were subjected to the further additional validation experiments. Here, we focused on the investigation of effect of overexpression SMS and SMOX on adipogenesis.

We firstly confirmed the successful overexpression of the hMSCs following the reporter gene GFP expressing via transfection using lentiviral system (Fig. 4a; 4b). With SMS and SMOX overexpressed, respectively, the relative expression level of adipogenic biomarkers, including CEBPA (CCAAT enhancer binding protein alpha), CEBPG (CCAAT enhancer binding protein gamma), FASN (fatty acid synthase), LPL (lipoprotein lipase), and PPARG (peroxisome proliferator activated receptor gamma) (Fig. 4c), was raised by SMOX more than SMS at transcriptional level. Protein level was brought into correspondence with the increase of PPARG gene expression (Fig. 4d). Compared with the negative control, the lipid droplets accumulation of SMOX overexpression was also more than that of SMS (Fig. 4e; 4f). Above all, SMOX overexpression enhanced adipogenesis at transcriptional, translational, and cellular phenotype level of lipid droplets accumulation more than SMS.

Here, we found SMOX overexpression fuels adipogenesis more than SMS using a lentivector expression system. Firstly, polyamine, ubiquitous positively charged amines, comprising agmatine, putrescine, spermidine, and spermine was essential for life, critical for adipogenesis from stem cell differentiation [14, 25–28]. Specially, spermidine was indispensable in differentiation of 3T3-L1 fibroblasts to adipocytes [29]. Exogenous polyamine also reciprocally regulated adipogenic differentiation [30]. Secondly, far fewer effects brought about by SMS enhancement was agreed with the study in transgenic mice displaying just slight increases in spermine and decreases in spermidine levels [31]. And it had proved that the expression of SMS was regulated primarily depending on substrate availability under the normal conditions and up-regulated when exposed to polyamines and their analogues [32]. As we known, spermidine was catalyzed by SMS to spermine, and spermine was inversely oxidized by SMOX to spermidine, 3-aminopropanal and hydrogen peroxide in polyamine pathway [33].

The predicted regulatory network between adipogenic biomarkers and amino acid metabolism

Here, except for BHMT2 for cysteine and methionine metabolism and ID2 for adipogenic biomarkers, we established the regulatory network between the other 16 DEGs of amino acid metabolism and 9 adipogenic biomarkers at transcriptional level using the Integrative Genomic Viewer (IGV) software (Fig. 5). It indicated that the DEGs of amino acid metabolism tightly related with the classical adipogenic biomarkers, such as AOC3-SMOX-PPARG-MAOA-LPL, AOC2-SMOX-PPARG-MAOA-LPL, ALDH4A1-SMS-SMOX-PPARG-MAOA-LPL, ALDH1A3-SMOX-PPARG-MAOA-LPL, ASS1-SMS-SMOX-PPARG, and so on. Especially, with SMS and SMOX overexpressed, PPARG was also partially confirmed to increase at transcriptional and translational level (Fig. 4c; 4d). In all, there was the potential regulatory network, and SMS-SMOX-PPARG was a potential and positive regulation signaling pathway. Although we found some evidence to support SMS-SMOX-PPARG signaling pathway working for amino acid metabolism during adipogenesis, further work on additional interaction mechanisms between the upstream and downstream in SMS-SMOX-PPARG signaling pathways should be detailed.

Conclusion

In summary, we firstly uncovered the transcriptional profiling of amino acid metabolism during adipogenesis by deep sequencing. We totally screened 17 DEGs for amino acid metabolism at 7, 14, 21, and 28 days during adipogenesis from hMSCs, especially with 13 up-regulated genes most prevalent in our adipogenic anecdote. Interestingly, the enforced expression of SMOX (spermine oxidase) responsible for polyamine metabolism in arginine and proline metabolism pathway facilitated adipogenesis more than SMS (spermine synthase). The established potential regulatory network further suggested that adipocyte differentiation tightly related with the basal metabolism of amino acid metabolism with the partially confirmed SMS-SMOX-PPARG signaling pathway. It would highlight the effect of adipogenesis on amino acid metabolism in adipocyte biology and provide the potential treatment strategy for the study of fat metabolic diseases.

Declarations

Funding

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Competing Interest

The authors declare that they have no conflict of interest.

Availability of data and materials

The data and materials that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions

XY conceived the idea, analyzed the RNA-Seq data and visualized data presentation, performed qRT-PCR and virus transfection experiment, and prepared the manuscript. XY, PW, and YG developed cell culture. XY and JYL carried out western blot. XY, XYX and JJX were in charge of the overall project. All authors read and approved the final manuscript.

Ethical statement

A 21-year-old non-osteoporotic healthy male volunteer was recruited from Affiliated Hospital of Jiujiang University. All the experimental manipulations performed following the relevant guidelines and regulations were verified and approved by the Medical Ethics Committee of Jiujiang University (Approved ID: 1-2013, February 20, 2013).

Consent to participate

The donor signed the written informed consent for the research following the ethical guidelines of Jiujiang University.

Consent for publication

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Figures

Figure 1

Adipogenic potential at 0 day (a), 7 days (b), 14 days (c), 21 days (d), and 28 days (e) from hMSCs. Scale bar was 20 μm.

Figure 2

Heat map of the DEGs for amino acid metabolism at 7, 14, 21, and 28 days during adipogenesis. The figures between brackets indicated the types of amino acid metabolism pathway.

0.48	0.40	0.36	0.40	MAOA	GO:0006576	cellular biogenic amine metabolic process
0.44	0.13	0.10	0.14	MAOA	GO:0006584	catecholamine metabolic process
0.21	0.01	0.00	0.02	MAOA	GO:0006005	serotonergic metabolic process
0.40	0.19	0.05	0.10	MAOA	GO:0007268	synaptic transmission
0.34	0.70	0.30	0.02	MAOA	GO:0007269	neurotransmitter secretion
0.32	0.59	0.32	0.60	MAOA	GO:0007610	behavior
0.25	0.72	0.67	0.16	MAOA	GO:0042135	neurotransmitter catabolic process
0.94	0.65	0.65	0.86	MAOA	GO:0042136	neurotransmitter biosynthetic process
0.55	0.47	0.43	0.47	MAOA	GO:0042420	dopamine catabolic process
0.00	0.00	0.00	0.00	MAOA	GO:0042421	small molecule metabolic process
0.00	0.04	0.00	0.00	MAOA	GO:0055114	oxidation-reduction process
0.38	0.31	0.28	0.32	ALDH1A3	GO:0002072	optic cup morphogenesis involved in camera-type eye development
0.22	0.15	0.13	0.26	ALDH1A3	GO:0002138	retinoic acid biosynthetic process
0.45	0.01	0.06	0.48	ALDH1A3	GO:0008152	metabolic process
0.28	0.22	0.20	0.22	ALDH1A3	GO:0021768	nucleus accumbens development
0.35	0.09	0.07	0.03	ALDH1A3	GO:0031076	embryonic camera-type eye development
0.23	0.05	0.01	0.11	ALDH1A3	GO:0042372	retinol metabolic process
0.35	0.21	0.15	0.21	ALDH1A3	GO:0042373	retinoic acid metabolic process
0.39	0.11	0.21	0.26	ALDH1A3	GO:0042374	retinol metabolic process
0.00	0.02	0.01	0.00	ALDH1A3	GO:0043065	positive regulation of apoptotic process
0.14	0.08	0.20	0.08	ALDH1A3	GO:0048048	embryonic eye morphogenesis
0.00	0.04	0.00	0.00	ALDH1A3	GO:0055114	oxidation-reduction process
0.16	0.11	0.09	0.11	ALDH1A3	GO:0060166	olfactory pit development
0.77	0.34	0.28	0.34	ALDH1A3	GO:0060324	face development
0.44	0.13	0.10	0.14	AOC2	GO:0006584	catecholamine metabolic process
0.69	0.08	0.36	0.60	AOC2	GO:0007601	visual perception
0.45	0.04	0.03	0.05	AOC2	GO:0009308	amine metabolic process
0.00	0.04	0.00	0.00	AOC2	GO:0055114	oxidation-reduction process
0.00	0.00	0.00	0.00	AOC3	GO:0006584	inflammatory response
0.00	0.00	0.00	0.00	AOC3	GO:0007155	cell adhesion
0.45	0.04	0.03	0.05	AOC3	GO:0009308	amine metabolic process
0.12	0.01	0.02	0.04	AOC3	GO:0046877	response to antibiotic
0.00	0.04	0.00	0.00	AOC3	GO:0055114	oxidation-reduction process
0.28	0.22	0.20	0.22	AOC3	GO:1802283	negative regulation of primary amine oxidase activity
0.28	0.53	0.49	0.53	SMS	GO:0006555	methionine metabolic process
0.56	0.65	0.58	0.40	SMS	GO:0006595	polyamine metabolic process
0.31	0.42	0.37	0.43	SMS	GO:0006596	polyamine biosynthetic process
0.48	0.40	0.36	0.40	SMS	GO:0006597	spermine biosynthetic process
0.11	0.66	0.64	0.62	SMS	GO:0034641	cellular nitrogen compound metabolic process
0.00	0.00	0.00	0.00	SMS	GO:0044281	small molecule metabolic process
0.16	0.40	0.09	0.40	ALDH4A1	GO:0006560	proline metabolic process
0.34	0.58	0.54	0.58	ALDH4A1	GO:0006561	proline biosynthetic process
0.28	0.53	0.16	0.53	ALDH4A1	GO:0006562	proline catabolic process
0.45	0.01	0.06	0.48	ALDH4A1	GO:0008152	metabolic process
0.16	0.40	0.09	0.40	ALDH4A1	GO:0010133	proline catabolic process to glutamate
0.22	0.47	0.43	0.47	ALDH4A1	GO:0019470	4-hydroxyproline catabolic process
0.11	0.66	0.64	0.62	ALDH4A1	GO:0034641	cellular nitrogen compound metabolic process
0.00	0.00	0.00	0.00	ALDH4A1	GO:0044281	small molecule metabolic process
0.00	0.04	0.00	0.00	ALDH4A1	GO:0055114	oxidation-reduction process
0.18	0.23	0.07	0.23	ASS1	GO:0000050	urea cycle
0.45	0.47	0.43	0.47	ASS1	GO:0006526	arginine biosynthetic process
0.59	0.84	0.78	0.70	ASS1	GO:0006652	cellular amino acid biosynthetic process
0.11	0.66	0.64	0.62	ASS1	GO:0034641	cellular nitrogen compound metabolic process
0.00	0.00	0.00	0.00	ASS1	GO:0044281	small molecule metabolic process
0.48	0.03	0.02	0.40	GGT1	GO:0002682	regulation of immune system process
0.29	0.08	0.16	0.15	GGT1	GO:0006508	proteolysis
0.67	0.40	0.11	0.56	GGT1	GO:0006520	cellular amino acid metabolic process
0.14	0.33	0.28	0.07	GGT1	GO:0006536	glutamate metabolic process
0.59	0.23	0.35	0.43	GGT1	GO:0006691	leukotriene metabolic process
0.21	0.47	0.36	0.63	GGT1	GO:0007749	glutathione metabolic process
0.05	0.09	0.32	0.21	GGT1	GO:0007750	glutathione biosynthetic process
0.28	0.22	0.20	0.22	GGT1	GO:0007751	glutathione catabolic process
0.21	0.01	0.00	0.02	GGT1	GO:0008005	xenobiotic metabolic process
0.69	0.40	0.11	0.56	GGT1	GO:0007263	spermatogenesis
0.48	0.40	0.36	0.40	GGT1	GO:0019344	cysteine biosynthetic process
0.19	0.00	0.04	0.01	GGT1	GO:0019369	arachidonic acid metabolic process
0.67	0.40	0.11	0.56	GGT1	GO:0019370	leukotriene biosynthetic process
0.00	0.00	0.00	0.00	GGT1	GO:0044281	small molecule metabolic process
0.26	0.05	0.09	0.26	GGT1	GO:0050727	regulation of inflammatory response
0.24	0.78	0.24	0.51	GGT1	GO:1901697	glutathione derivative biosynthetic process
0.29	0.08	0.16	0.15	GGT5	GO:0006508	proteolysis
0.67	0.40	0.11	0.55	GGT5	GO:0006520	cellular amino acid metabolic process
0.59	0.23	0.35	0.43	GGT5	GO:0006691	leukotriene metabolic process
0.21	0.47	0.36	0.63	GGT5	GO:0007749	glutathione metabolic process
0.05	0.09	0.32	0.21	GGT5	GO:0007750	glutathione biosynthetic process
0.00	0.00	0.00	0.00	GGT5	GO:0008005	inflammatory response
0.19	0.00	0.04	0.01	GGT5	GO:0019369	arachidonic acid metabolic process
0.77	0.40	0.53	0.61	GGT5	GO:0019370	leukotriene biosynthetic process
0.00	0.00	0.00	0.00	GGT5	GO:0044281	small molecule metabolic process
0.14	0.08	0.06	0.02	GLUL	GO:0001504	neurotransmitter uptake
0.14	0.33	0.28	0.07	GLUL	GO:0006536	glutamate metabolic process
0.55	0.47	0.43	0.47	GLUL	GO:0006538	glutamate catabolic process
0.38	0.31	0.28	0.32	GLUL	GO:0006542	glutamine biosynthetic process
0.35	0.19	0.06	0.19	GLUL	GO:0006807	nitrogen compound metabolic process
0.80	0.19	0.05	0.10	GLUL	GO:0007268	synaptic transmission
0.04	0.02	0.00	0.05	GLUL	GO:0008283	cell proliferation
0.59	0.44	0.38	0.70	GLUL	GO:0008652	cellular amino acid biosynthetic process
0.43	0.01	0.01	0.00	GLUL	GO:0009749	response to glucose
0.04	0.07	0.01	0.07	GLUL	GO:0030224	positive regulation of insulin secretion
0.11	0.66	0.64	0.62	GLUL	GO:0034641	cellular nitrogen compound metabolic process
0.00	0.00	0.00	0.00	GLUL	GO:0044281	small molecule metabolic process
0.00	0.00	0.00	0.00	GLUL	GO:0050679	positive regulation of epithelial cell proliferation
0.12	0.18	0.25	0.25	GLUL	GO:0051260	protein homodimerization
0.20	0.21	0.02	0.10	GLUL	GO:0051968	positive regulation of synaptic transmission, glutamatergic
0.96	0.65	0.58	0.40	SMOX	GO:0006595	polyamine metabolic process
0.31	0.42	0.37	0.43	SMOX	GO:0006596	polyamine biosynthetic process
0.38	0.31	0.28	0.32	SMOX	GO:0006598	polyamine catabolic process
0.21	0.01	0.00	0.02	SMOX	GO:0008005	xenobiotic metabolic process
0.11	0.66	0.64	0.62	SMOX	GO:0034641	cellular nitrogen compound metabolic process
0.00	0.00	0.00	0.00	SMOX	GO:0044281	small molecule metabolic process
0.38	0.31	0.28	0.32	SMOX	GO:0046208	spermine catabolic process
0.00	0.04	0.00	0.00	SMOX	GO:0055114	oxidation-reduction process
0.73	0.63	0.08	0.64	ADH1A	GO:0006066	alcohol metabolic process
0.21	0.11	0.03	0.26	ADH1A	GO:0006069	ethanol oxidation
0.21	0.01	0.00	0.02	ADH1A	GO:0008005	xenobiotic metabolic process
0.18	0.21	0.14	0.06	ADH1A	GO:0017144	drug metabolic process
0.00	0.00	0.00	0.00	ADH1A	GO:0044281	small molecule metabolic process
0.00	0.04	0.00	0.00	ADH1A	GO:0055114	oxidation-reduction process
0.21	0.11	0.03	0.26	ADH1B	GO:0006069	ethanol oxidation
0.21	0.01	0.00	0.02	ADH1B	GO:0008005	xenobiotic metabolic process
0.00	0.00	0.00	0.00	ADH1B	GO:0044281	small molecule metabolic process
0.00	0.04	0.00	0.00	ADH1B	GO:0055114	oxidation-reduction process
0.21	0.11	0.03	0.26	ADH1C	GO:0006069	ethanol oxidation
0.21	0.01	0.00	0.02	ADH1C	GO:0008005	xenobiotic metabolic process
0.00	0.00	0.00	0.00	ADH1C	GO:0044281	small molecule metabolic process
0.00	0.04	0.00	0.00	ADH1C	GO:0055114	oxidation-reduction process
0.35	0.78	0.41	0.47	BHMT2	GO:0009086	methionine biosynthetic process
0.69	0.40	0.11	0.56	BHMT2	GO:0030229	methylation
0.00	0.00	0.00	0.00	CYP1B1	GO:0015525	angiogenesis
0.34	0.08	0.06	0.08	CYP1B1	GO:0006725	cellular aromatic compound metabolic process
0.21	0.01	0.00	0.02	CYP1B1	GO:0008005	xenobiotic metabolic process
0.69	0.40	0.11	0.56	CYP1B1	GO:0007601	visual perception
0.03	0.01	0.00	0.04	CYP1B1	GO:0008202	steroid metabolic process
0.12	0.02	0.04	0.06	CYP1B1	GO:0009404	toxin metabolic process
0.80	0.19	0.06	0.80	CYP1B1	GO:0009036	response to toxic substance
0.06	0.05	0.17	0.02	CYP1B1	GO:0018125	sterol metabolic process
0.19	0.00	0.04	0.01	CYP1B1	GO:0019369	arachidonic acid metabolic process
0.84	0.23	0.41	0.76	CYP1B1	GO:0019373	epoxygenase P450 pathway
0.01	0.00	0.01	0.00	CYP1B1	GO:0043542	endothelial cell migration
0.00	0.00	0.00	0.00	CYP1B1	GO:0044281	small molecule metabolic process
0.00	0.04	0.00	0.00	CYP1B1	GO:0055114	oxidation-reduction process
0.18	0.29	0.24	0.29	CYP1B1	GO:0061298	retina vasculature development in camera-type eye
0.16	0.40	0.09	0.11	CYP1B1	GO:0071603	endothelial cell-cell adhesion
0.61	0.16	0.33	0.36	CYP1B1	GO:0097267	omega-hydroxyase P450 pathway
0.00	0.51	0.33	0.07	GPX3	GO:0006979	response to oxidative stress
0.38	0.31	0.28	0.32	GPX3	GO:0006982	response to lipid hydroperoxide
0.35	0.24	0.16	0.56	GPX3	GO:0042744	hydrogen peroxide catabolic process
0.11	0.70	0.19	0.30	GPX3	GO:0051289	protein homodimerization
0.00	0.04	0.00	0.00	GPX3	GO:0055114	oxidation-reduction process

Figure 3

GO analysis of the DEGs for amino acid metabolism during adipogenesis.

Figure 4

Gene function of SMS and SMOX during adipogenesis. (a) Fluorescence intensity after RNAi. Scale bar was 50 μ m. (b) The relative expression level of SMS and SMOX after RNAi. (c) The relative expression level of adipogenic biomarkers with SMS and SMOX overexpressing, respectively. (d) Protein level via western blot. (e) Adipogenic potential of hMSCs via Oil Red O staining. Scale bar was 20 μ m. (f) Quantification of Oil Red O during adipogenesis.

Figure 5

Regulatory networks between our DEGs and adipogenic biomarkers. Our DEGs were included in amino metabolism. The labeling of the selected genes were added manually using the Integrative Genomic Viewer (IGV) software. SMS and SMOX were colored yellow, and the classic adipogenic biomarkers were colored green.