Matrix metalloproteinase activity is essential for proper extracellular matrix remodeling that takes place during adipose tissue formation. Four tissue inhibitors of matrix metalloproteinases (TIMPs) regulate their activity. However, the role of TIMPs in adipocyte differentiation is poorly understood. We found that the expression of all TIMPs was modified during adipocyte differentiation, but that of TIMP-3 was distinguished by its extreme down-regulation. TIMP-3 expression was closely linked to the differentiation process. Indeed, it remained low during the adipocyte differentiation but increased when cell differentiation was prevented. We identified the transcription factor Sp1 as being responsible for the regulation of TIMP-3 expression during adipocyte differentiation. Overexpression of TIMP-3 reduced adipocyte differentiation, underlining its active role in this process. TIMP-3 overexpression decreased the expression of the early and obligate key inducers of adipogenesis Krüppel-like factor 4 (Klf4), early growth response 2 (Egr2/Krox20), and CAAT/enhancer-binding protein β (C/EBPβ). Our results indicate that during preadipocyte differentiation, the Sp1-dependent decrease in TIMP-3 expression is required for the successful implementation of the adipocyte differentiation program.

Development of obesity is associated with extensive modifications of the composition and architecture of the adipose tissue. These events necessitate dynamic changes of cell-matrix interactions and extracellular matrix remodeling that are made possible by the alteration of pericellular proteolytic activities. Normal turnover of the extracellular matrix is regulated by the opposing activities of proteinases and their inhibitors (1, 2). Matrix metalloproteinases (MMPs)4 are a family of >20 secreted zinc-dependent proteinases involved in the extracellular matrix degradation, the release of sequestered growth factors, and the cleavage of cell surface receptors (3). Several lines of evidence suggest a potential role of MMPs in the development of adipose tissue. Modulation of MMP expression was reported in adipose tissue of mice with nutritionally induced obesity as well as genetically obese mice (4–6). Treatment of mice with a large spectrum MMP inhibitor reduces the development of adipose tissue (7). When subjected to high fat diet, mice deficient in MMP-3, -11, and -19 develop more adipose tissue than control mice (8–10). Contrarily, mice deficient in MMP-2 and membrane type-1 MMP show a lipodystrophic phenotype (11, 12). MMP-9 deficiency does not influence adiposity (13). MMP activity is regulated by four tissue inhibitors of MMPs (TIMPs) whose expression is regulated during adipose tissue development (4, 6). Only the role of TIMP-1 in obesity has been studied. TIMP-1-deficient mice are protected from obesity (14).

Few data are available concerning the role of MMPs and TIMPs in adipocyte differentiation. The expression of most MMPs is modulated during the in vitro differentiation of mouse preadipocyte cell lines (4, 6, 15, 16). The importance of the subtle regulation of MMPs for the achievement of adipose differentiation is emphasized by the effects of their inhibition. Depending on their nature, synthetic MMP inhibitors were reported to up- or down-regulate adipogenesis, and inhibition of MMP-2 and -9 by neutralizing antibodies was shown to reduce differentiation (4, 6, 15–17). The expression of all TIMPs is also modulated during adipogenesis, and addition of TIMP-1 was shown to accelerate lipogenesis in 3T3-L1 cultures (4, 6, 15). It is thus conceivable that the regulation of TIMP expression is involved in the control of adipogenesis. However, no direct experimental evidence is yet available to support this hypothesis. We investigated the regulation of TIMPs during adipogenesis of 3T3-L1 cells, and we found that TIMP-3 expression was strongly reduced compared with that of other TIMPs. This observation, when combined with results showing that TIMP-3 deficiency leads to enhanced adipose reconstitution during mammary involution (18), suggests that TIMP-3 down-regulation is necessary for adipogenesis. Consequently, to test this hypothesis, we studied the regulation and role of TIMP-3 in adipocyte differentiation.

We showed that TIMP-3 expression is regulated by the transcription factor Sp1. A low level of TIMP-3 is necessary for adipocyte differentiation, and adipocyte differentiation maintains a low level of TIMP-3. Reciprocally, a high level of TIMP-3 impedes adipocyte differentiation. The results show that...
TIMP-3 is involved in the regulation of the essential early inductors of adipogenesis, i.e. Krüppel-like factor 4 (Klf4), early growth response 2 (Egr2/Krox20), and CAAT/enhancer-binding protein β (C/EBPβ) and indicate that TIMP-3 down-regulation is mandatory for the proper implementation of the adipocyte differentiation program.

EXPERIMENTAL PROCEDURES

Materials

Rolipram, adenosine 3',5'-cyclic phosphorothioate-Rp, dexamethasone (Dex), 3-isobutyl-1-methylxanthine (Mix), H89, WP631, insulin, and Ru 486 were from Sigma-Aldrich. Dibutyryl-cAMP was from Calbiochem. Anti-TIMP-3 and antilamin B1 antibodies were from Abcam. Anti-Sp1 (PEP 2 recognizing both p95 and p106 isoforms), anti-C/EBPβ (Δ198) and anti-actin (C-11-R) antibodies were from Santa Cruz Biotechnologies (Santa Cruz, CA). Primers and small interfering RNA were from Eurogentec (Anger, France).

Cell Culture, Differentiation, and Transfection

Swiss 3T3-L1 preadipocytes were routinely cultured in Dulbecco’s modified Eagle’s medium with 4 mM l-glutamine, 4.5 g/liter glucose, 0.11 g/liter sodium pyruvate, and supplemented with 10% fetal bovine serum plus antibiotics. Two days after confluence (d−2), preadipocytes were staged to differentiate by changing the medium to one containing the induction mixture (0.1 mM Dex, 500 mM Mix, and 2 mM insulin). After 48 h (d0), the medium was removed and replaced by Dulbecco’s modified Eagle’s medium containing 2 mM insulin only. Human subcutaneous preadipocytes and mouse embryonic fibroblasts were obtained as already described (19, 20). Human visceral preadipocytes were from Lonza (Levallois-Perret, France). For differentiation and gene reporter experiments, preadipocytes were transiently transfected by electroporation using Amaxa Nucleofector II (Lonza) and Icafeclin 441 (Eurogentec), respectively.

Plasmids

The human TIMP-3 cDNA coding sequence (hTIMP-3) was cloned using total RNA from THP-1 cells. The PCR product was cloned into pCR2.1 vector (Invitrogen) and sequenced then inserted into pcDNA3 vector. Full-length mouse TIMP-3 cloned into the expression vector pCMVSport6 was from Invitrogen. Sp1 expression vector was from Dr. R. Planells (UMR INRA 1260/INSERM 476, Université de la Méditerranée, Marseille, France). Mouse TIMP-3 promoter from nucleotides −2555 to +290 (ATG at position 316) was PCR-amplified from genomic DNA from 3T3-L1 cells, cloned into pCR2.1 vector, sequenced, then inserted into PGL3 Basic vector (Promega) for luminescence experiments. Sp1 luciferase reporter vector was from Panomics (Fremont, CA). CRE-SEAP reporter vector, which encodes the secreted human placental alkaline phosphatase (SEAP) reporter driven by a promoter containing multiple cAMP-responsive elements (pCRE-SEAP), was from Y. Durocher (Biotechnology Research Institute, National Research Council Canada, Montreal, Canada).

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Gene Reporter Experiments

Luciferase Assay—Cells were cotransfected with firefly luciferase reporter vectors and with Renilla luciferase control vector (PGL4 SV40 from Promega). Firefly and Renilla luciferase activities were measured with a luminometer. The activity of promoters is given as the ratio of the arbitrary units of luminescence of firefly versus Renilla luciferase.

SEAP Assay—Cells were transfected with the reporter plasmid pCRE-SEAP. The level of SEAP protein in the medium was assayed by degradation of para-nitrophenyl phosphate. Briefly, following the incubation period, 20 μl of culture medium was transferred to a 96-well plate and mixed with 80 μl of SEAP assay solution containing 12.5 mM para-nitrophenyl phosphate, 0.4 mM MgCl₂, and 0.4 mM diethanolamine, pH 9.8. After a 15-min incubation, absorbance was read at 410 nm.

Real Time PCR Analysis

Total or nuclear RNA was extracted using the RNEasy mini kit (Qiagen) or the cytoplasmic and nuclear RNA purification kit (Norgen Biotek Corp., Thorold, ON, Canada), respectively, and DNase I (Qiagen)-treated. Real time PCR was performed as described previously (21) on a 7300 Real Time PCR system (Applied Biosystems) using the Mesa Green MasterMix (Eurogentec). Primer sequences are available upon request. The relative amounts of the different mRNAs were quantified by using the comparative CT method (2−△△CT). 36B4 and 18S were used as housekeeper transcripts for mouse and human cells, respectively.

Cell Proliferation and Caspase Activity

Cell number was determined using the CyQUANT cell proliferation assay kit following the manufacturer’s procedures (Invitrogen). Caspase activity was detected in living cells using a multicaspase staining kit (PromoKine, Heidelberg, Germany) following the manufacturer’s guidelines. Active caspases in living cells were labeled with cell-permeable fluorescein isothiocyanate-conjugated caspase inhibitor VAD-fluoromethyl ketone. Fluorescence was measured with a fluorescent plate reader (HiDex Chameleon); values are in arbitrary fluorescence units/cell number.

Statistical Analyses

All experiments were performed at least in triplicate and at least three times. All data are expressed as the mean ± S.D. Treatments were compared with their respective controls, and significant differences among the groups were determined using an unpaired Student’s t test. A value of p < 0.05 was taken as an indication of statistical significance.

RESULTS

TIMP-3 Expression Is Drastically Reduced during Adipocyte Differentiation—As expected, mRNA levels of adipogenic markers sterol regulatory element-binding protein 1c (SREBP-1c), peroxisome proliferator-activated receptor γ (PPARγ), and leptin gradually increased during 3T3-L1 differentiation (Fig. 1A), whereas those of the preadipocyte factor 1 (Pref1) decreased considerably, during the staging period (between
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FIGURE 1. Expression of TIMPs during adipocyte differentiation. A and B, real time PCR analysis of mRNAs levels of adipocyte markers (SREBP-1c, PPARγ, and leptin), preadipocyte marker (Pref1) (A), and TIMP-1, -2, -3, -4 during adipocyte differentiation of 3T3-L1 cells (B). Inset, Western blot of TIMP-3 contained in the extracellular matrix. C, real time PCR analysis of TIMP-3 mRNA levels of mouse embryonic fibroblasts (MEF), human visceral (VIS) and subcutaneous (SC) preadipocytes before (d–2) and after 2 days (d0) of treatment with the induction mixture. Days of analysis are indicated below graphs. Error bars, S.D.

d–2 and d0) triggered by the treatment of confluent preadipocytes with the induction mixture (Dex, Mix, and insulin). The mRNA levels of all TIMPs were reduced during the staging period (Fig. 1B). During the differentiation process (after d0), mRNA levels of TIMP-1, -2, and -3 remained low, whereas those of TIMP-4 increased back to their initial levels. The decrease in TIMP-3 mRNA levels was substantial compared with that of other TIMPs. Moreover, it was also observed in mouse embryonic fibroblasts and in human visceral and subcutaneous preadipocytes following the stimulation with the induction mixture (Fig. 1C), indicating that this process is not limited to 3T3-L1 preadipocytes. We thus focused our study on the regulation and role of TIMP-3 in adipogenesis.

Adipocyte Differentiation Maintains Low Level of TIMP-3—The decrease in TIMP-3 and Pref1 mRNA levels occurred during the staging period. Then, during the differentiation phase, cells were no longer subjected to the induction mixture, yet the mRNA levels of TIMP-3 and Pref1 remained low (Fig. 1B). We analyzed whether the maintenance of these low levels of mRNA was the consequence of the adipocyte differentiation process. Cells were treated for differentiation in the presence or absence of glucose. As expected, in the presence of glucose the process of adipocyte differentiation occurred normally (Fig. 1A), and TIMP-3 and Pref1 mRNA levels decreased during the staging period and remained low during the differentiation phase (Figs. 1A and 2). Without glucose, TIMP-3 and Pref1 mRNA levels decreased also during the staging period, but then they increased gradually during the differentiation phase (Fig. 2). Similar results were obtained in the absence of insulin (data not shown). As expected, without glucose or insulin, terminal differentiation was blocked as evidenced by the lack of accumulation of lipid droplets in cells and the increase in SREBP-1c and PPARγ mRNA levels (data not shown). This result suggests that the treatment with the induction mixture is necessary for the reduction of TIMP-3 and Pref1 mRNA levels and that the differentiation process (or the glucose uptake) is necessary to maintain low levels of these mRNA over time.

TIMP-3 Down-regulation Promotes Adipogenesis—To characterize further the role of TIMP-3 in adipogenesis, 3T3-L1 cells were transfected with hTIMP-3 expression vector and submitted to the adipogenic treatment. Overexpression of hTIMP-3 increased the global caspase activity modestly (+12%; p = 0.001) with no obvious change in cell viability as the clonal expansion of 3T3-L1 cells triggered by the induction mixture was not altered (Fig. 3A and B). After 3 days of differentiation, 3T3-L1 cells expressing hTIMP-3 accumulated less lipids (Fig. 3C) and expressed significantly less SREBP-1c (p = 0.0008), PPARγ (p = 0.0117), and leptin (p = 0.005) than cells transfected with an empty plasmid (Fig. 3D), indicating that the differentiation process is impaired by hTIMP-3 overexpression.

FIGURE 2. Adipocyte differentiation maintains low level of TIMP-3. 3T3-L1 cells cultured in the absence or presence of glucose were submitted to the adipogenic treatment. TIMP-3 (left) and Pref1 (right) mRNA levels were analyzed by real time PCR. Days of analysis are indicated below graphs. *, significant versus the situation with glucose (p < 0.007). Error bars, S.D.
As a logical result of inhibition of adipogenesis, the mRNA levels of endogenous TIMP-3 and Pref1 were significantly higher when hTIMP-3 was overexpressed ($p = 0.0013$ and $p = 0.03$, respectively). Similar inhibition of differentiation was obtained with mouse TIMP-3 overexpression or after the transfection of a construct (hTIMP-3 coding sequence fused with the 3'-untranslated region of mouse TIMP-3) that gives a low expression level of hTIMP-3 (approximately 7-fold less than the amount shown in Fig. 3C) (data not shown). This result underlines the importance of the loss of TIMP-3 for an efficient adipose conversion.

**Transcriptional Events Are Involved in TIMP-3 Reduction**—As a result of the finding that TIMP-3 overexpression inhibited the accumulation of lipids in 3T3-L1 cells, we next sought to study its regulation during adipogenesis. The drastic decrease in TIMP-3 mRNA levels occurred after the beginning of the adipogenic treatment, suggesting that the induction mixture is directly responsible for this decrease. A 24-h treatment with Mix and Dex, alone or in combination, significantly reduced TIMP-3 mRNA levels (Fig. 4A). Insulin was without effect (data not shown). The combination of Mix and Dex also reduced the expression of all other TIMPs mRNA. However, when used alone, Mix and Dex modulated the expression of TIMP-1, -2, and -4 in divergent ways. Mix increased TIMP-1, reduced TIMP-4, and did not modify TIMP-2, and Dex slightly increased TIMP-4. Furthermore, this short treatment with Mix and Dex did not alter PPARγ mRNA levels. These results prove the specific effect of Mix and Dex on TIMP-3 mRNA levels.

Mix and Dex, alone or in combination, significantly reduced the activity of TIMP-3 promoter ($p \approx 0.0014$) measured using luciferase reporter gene (Fig. 4B). Expectedly, the amount of TIMP-3 nuclear pre-mRNA was also reduced by Mix and Dex (Fig. 4C). These results suggest that these compounds alter the transcription of TIMP-3 gene.

**Mode of Action of Dex and Mix on TIMP-3 Expression**—The inhibitory effect of Dex on TIMP-3 expression was abolished by the glucocorticoid receptor antagonist Ru 486, indicating the involvement of glucocorticoid receptors (Fig. 5). The increase in cAMP is responsible for the Mix-induced inhibition of TIMP-3 expression as rolipram (a phosphodiesterase inhibitor) and a stable cAMP analog (dibutyryl-cAMP) acted like Mix (Fig. 5). The stimulation of cAMP-dependent pathway by Mix was confirmed by the increased expression of a secreted alkaline phosphatase under the transcriptional control of CRE elements (Fig. 5B, inset). However, two inhibitors of protein kinase
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A (H89 and adenosine 3′,5′-cyclic phosphorothioate-Rp) did not reverse the effect of Mix on TIMP-3 (data not shown), suggesting that protein kinase A is not involved in the effect of Mix.

To investigate how Dex and Mix reduced TIMP-3 expression, cells were incubated for 24 h with a selection of inhibitors or activator that could interfere with their effects, and TIMP-3 mRNA levels were measured. Inhibitors of c-Jun N-terminal kinase (JNK), protein kinase C, MAPK kinase, p38 MAPK, AMP-activated protein kinase neither mimicked nor antagonized the effect of Mix and Dex (data not shown).

**(Sp1-driven TIMP-3 Transcription Is Reduced by Mix and Dex)**

Sp1-driven TIMP-3 transcription was measured 24 h later. Cells were untreated (Cont) or treated for 24 h with Dex or Mix.

**A**. Western blot analysis of nuclear Sp1 from 3T3-L1 cells untreated (Cont) or treated for 24 h with Dex and Mix. B. Sp1 expression vector, and luciferase activity was measured 24 h later. C. Cells were untreated (Cont) or treated for 24 h with Dex or Mix in the absence or presence of WP631 (100 nM), and TIMP-3 mRNA levels were analyzed by real-time PCR. *Error bars*, S.D.

**FIGURE 6. Sp1-driven TIMP-3 transcription is reduced by Mix and Dex.** A, Western blot analysis of nuclear Sp1 from 3T3-L1 cells untreated (Cont) or treated for 24 h with Dex and Mix. B, same as Fig. 4B except that cells were transfected with Sp1 luciferase reporter vector. C, cells were cotransfected with Sp1 or TIMP-3 luciferase reporter vector together with empty plasmid or Sp1 expression vector, and luciferase activity was measured 24 h later. D, cells were untreated (Cont) or treated for 24 h with Dex or Mix in the absence or presence of WP631 (100 nM), and TIMP-3 mRNA levels were analyzed by real-time PCR. Error bars, S.D.

**FIGURE 7. Adipocyte differentiation maintains a low Sp1 transcriptional activity.** 24 h after the transfection with Sp1 luciferase gene reporter, 3T3-L1 cells were treated for 48 h with the induction mixture and then the cells were incubated for 24 h in a complete medium with insulin (Ins), without insulin (−Ins), or without glucose (−Glu), and luciferase activity was measured. *Error bars*, S.D.

Adipocyte Differentiation Maintains a Low Sp1 Transcriptional Activity—During the differentiation process, TIMP-3 mRNA levels remain low even in the absence of Dex and Mix. Contrarily, in the absence of differentiation, TIMP-3 mRNA levels increase slowly after their initial reduction (Fig. 2), suggesting that the differentiation process modifies the way TIMP-3 is regulated. To explain this regulation, we analyzed the evolution of Sp1 transcriptional activity following the induction of differentiation. Cells expressing Sp1 luciferase reporter gene were treated for 48 h with the induction mixture followed by an incubation of 24 h in the presence or absence of insulin or glucose. In the absence of insulin or glucose, the Sp1 activity was significantly higher (+47%, *p* < 0.0001 and +34%, *p* = 0.0037, respectively) than that measured in the presence of insulin and glucose (Fig. 7), suggesting that the process of differentiation (or glucose uptake) represses the transcriptional activity of Sp1, which may explain the low expression level of TIMP-3 during the course of differentiation.

**TIMP-3 Expression Modulates the Differentiation Program**—In an attempt to explain how hTIMP-3 overexpression reduces adipogenesis, we analyzed the expression of the early transcription factors essential for adipocyte differentiation. We focused on the study on C/EBPβ and on the transcription factors involved in its expression, *i.e.*, Klf4 and Egr2/Krox20. The expression of all of these factors increased transiently after the addition of the induction mixture (Fig. 8A). The increase in Klf4, Egr2/Krox20,
and C/EBPβ mRNA levels was significant as soon as 30 min after the addition of the induction mixture (p < 0.003). Overexpression of hTIMP-3 did not alter the basal mRNA levels of these transcription factors but significantly inhibited the increase of Klf4 (−20%, p = 0.02), Egr2/Krox20 (−15%, p = 0.03), and C/EBPβ (−20%, p = 0.01) mRNA levels triggered by the induction mixture (Fig. 8B). Furthermore, the increase in C/EBPβ protein that was significantly detectable 4 h after the addition of the induction mixture (Fig. 8C) was largely prevented by hTIMP-3 overexpression (Fig. 8D).

DISCUSSION

In this work, we investigated the regulation and role of TIMP-3 during preadipocyte differentiation. We show that the expression of TIMP-3 determines adipocyte differentiation. Of the four TIMPs, TIMP-3 undergoes the most drastic alteration of its expression after the induction of adipocyte differentiation of 3T3-L1 cells. Indeed, TIMP-3 mRNA levels decrease after the addition of the induction mixture and then stay low during the adipocyte conversion. A similar regulation of TIMP-3 mRNA was already mentioned in the same model (15). In addition, we show that this regulation is a general feature of all adipocyte differentiation models that we tested (human subcutaneous and visceral preadipocytes and mouse embryonic fibroblasts) and that is followed by a reduction of the TIMP-3 amount detectable in the extracellular matrix.

The loss of TIMP-3 expression is triggered by the addition of the differentiation mixture and precedes the acquisition of adipose phenotype (accumulation of cytoplasmic lipid droplets), indicating that components of the differentiation mixture are responsible for this process. We indeed show that Mix and Dex, which are the basic components of all adipocyte differentiation cocktails, are responsible for the decrease in TIMP-3 expression. Both compounds decrease TIMP-3 expression primarily by reducing its gene transcription. Hypermethylation of TIMP-3 promoter is by far the most studied process by which TIMP-3 gene transcription could be reduced. However, in our hands, two different DNA methylation inhibitors (azacytidine and RG-108) did not reduce the loss of TIMP-3 mRNA triggered by Dex and Mix, suggesting that in our situation, hypermethylation of TIMP-3 promoter is not involved in the down-regulation of TIMP-3. We compile arguments in favor of the role of the transcription factor Sp1 in the Mix- and Dex-dependent regulation of TIMP-3. As already described, we show that the transcriptional activity of Sp1 is reduced by Mix and Dex (25, 28) and overexpression of Sp1 increases TIMP-3 promoter activity (28). An inhibitor of Sp1-activated transcription prevents the effect of Mix and Dex on TIMP-3 expression. All our data converge to indicate that the reduction of Sp1 transcriptional activity induced by Dex and Mix participates in the decrease in TIMP-3 expression.

It is noteworthy that TIMP-3 mRNA levels remain low during the adipocyte differentiation. However, when differentiation is impaired, by the absence of glucose or insulin, TIMP-3 mRNA levels rise back over time. The transcriptional activity of Sp1 was regulated similarly: in the absence of glucose or insulin, Sp1 activity is higher than in the situation of normal differentiation. These results suggest that the transcriptional activity of Sp1 and consequently the expression of TIMP-3 are intimately linked to the differentiation capacity of the cells. A decrease in Sp1 level early in the differentiation program was previously shown to derepress C/EBPα gene (25) which likely facilitates adipocyte differentiation.

The necessity of the reduction of preadipocyte TIMP-3 expression for an efficient adipogenesis is underscored by the fact that overexpression of TIMP-3, even when it is very low, significantly prevents adipocyte differentiation. To our knowledge, the direct role of preadipocyte TIMPs expression, in the first steps of preadipocyte differentiation, was never addressed before. Our work did not address the effect of the other preadipocyte TIMPs on the regulation of adipogenesis. However, TIMP-3 is the only TIMP that binds strongly to the extracellular matrix, which confines its action close to the cells that produce it. It is therefore reasonable to assume that in adipose tissue, an altered expression of preadipocyte TIMP-3 is more efficient to affect their differentiation than an altered expression of any other preadipocyte TIMPs.

It was described that TIMP-3 overexpression triggered apoptosis in several cell lines (29–32). We show that it causes a modest caspase activation in 3T3-L1 cells that does not result in
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a long term cell mortality as confirmed by the absence of effect on postclonal expansion, a phenomenon reported to be necessary for the differentiation of 3T3-L1 cells (33). It is known that during adipocyte differentiation, the early expression of C/EBPα induces C/EBPα and PPARγ (34), which are responsible for the coordinated regulation of expression of several genes leading to terminal differentiation. We show that TIMP-3 overexpression impedes the expression of the transcription factor C/EBPα triggered by the induction mixture. The expression of the transcription factors Klf4 and Egr2/Krox20, which were recently described as essential for the induction of C/EBPα expression and adipogenesis (35–37), was also reduced by TIMP-3 overexpression, suggesting that TIMP-3 blocks the very early steps of the adipogenesis program.

Further studies are needed to identify the mechanisms by which TIMP-3 modulates the expression of Klf4 and Egr2/Z/Krox20. These mechanisms could be related to MMPs activity and the composition of the extracellular matrix because TIMP-3 inhibits at least MMP-2 and MMP-9 (38), both involved in adipocyte differentiation (4, 6, 16). Furthermore, TIMP-3 not only inhibits MMPs but is also capable of inhibiting members of two groups within the adamalysin family: the ADAMs (a disintegrin and metalloproteinase domain) and ADAMTSs (ADAM with thrombospondin-like repeats). However, the implication of mechanisms that involve the inhibition of ADAM-17 by TIMP-3 would be in contradiction with previous results showing that adipocyte differentiation is increased when ADAM-17 is knocked down (17). In conclusion, our work suggests that the Sp1-dependent reduction of TIMP-3 expression at the beginning of preadipocyte differentiation process, followed by a low steady-state level of this molecule, is necessary for the proper expression of C/EBPα leading to the correct implementation of the differentiation program and to the acquisition of mature adipocyte phenotype.

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