Leukemia Inhibitory Factor and Its Receptor Promote Adipocyte Differentiation via the Mitogen-activated Protein Kinase Cascade*

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Extracellular factors and intracellular signaling pathways involved in early events of adipocyte differentiation are poorly defined. It is shown herein that expression of leukemia inhibitory factor (LIF) and LIF receptor is developmentally regulated during adipocyte differentiation. Preadipocytes secrete bioactive LIF, and an antagonist of LIF receptor inhibits adipogenesis. Genetically modified embryonic stem (ES) cells combined with culture conditions to commit stem cells into the adipocyte lineage were used to examine the requirement of LIF receptor during in vitro development of adipose cells. The capacity of embryoid bodies derived from ES cells to undergo adipocyte differentiation is dramatically reduced. LIF addition stimulates adipocyte differentiation of Ob1771 and 3T3-F442A preadipocytes and that of peroxisome proliferator-activated receptor γ ligand-treated mouse embryonic fibroblasts. Expression of the early adipogenic transcription factors C/EBPβ and C/EBPδ is rapidly stimulated following exposure of preadipose cells to LIF. The selective inhibitors of mitogen-activated protein kinase kinase, i.e. PD98059 and U0126, inhibit LIF-induced C/EBP gene expression and prevent adipocyte differentiation induced by LIF. These results are in favor of a model that implicates stimulation of LIF receptor in the commitment of preadipocytes to undergo terminal differentiation by controlling the early expression of C/EBPβ and C/EBPδ genes via the mitogen-activated protein kinase cascade.

The adipocyte differentiation program is controlled by the sequential expression of recently identified transcription factors. Members of the CCAAT/enhancer binding protein (C/EBP) family and peroxisome proliferator-activated receptor (PPAR) families are trans-acting nuclear factors playing a regulatory role in the differentiation of preadipocytes into adipocytes (1, 2). PPARγ2 is predominantly expressed in adipose tissue and plays a critical role in the adipocyte differentiation process (3, 4). Expression of C/EBPβ and C/EBPδ genes is not restricted to adipose tissue but is induced early and transiently during the program of adipocyte differentiation. Expression of C/EBPβ and C/EBPδ decreases following adipocyte maturation, whereas expression of C/EBPα and PPARγ2 gene is induced (5). C/EBP-binding sites have been identified in the PPARγ2 promoter, and it has been recently shown that C/EBP proteins directly control transcription from the PPARγ2 promoter (6). The adipogenic role of C/EBPβ and C/EBPδ has been previously demonstrated. Their ectopic expression in fibroblasts leads, in the presence of adipogenic hormones, to the adipocyte phenotype (7–9). Finally, the generation by homologous recombination of C/EBPβ−/− and C/EBPδ−/− mice has clearly established the essential role of these two C/EBPs for the acquisition of adipocytes both in vitro and in vivo (10). However, the extracellular factors and the intracellular signaling pathways involved in the regulation of C/EBPβ and C/EBPδ expression in preadipose cells are poorly defined. During the course of our investigation to identify extracellular factors that regulate early events in adipocyte differentiation, secretion of leukemia inhibitory factor (LIF) by preadipocytes was observed. LIF is known to induce differentiation of the murine myeloid leukemia cell line M1, to maintain pluripotent embryonic stem (ES) cells (11, 12), and to modulate stem cell differentiation and cell type functions in vitro and in vivo (13). LIF and the related cytokines cardiophrin (CT-1) and ciliary neurotrophic factor (CNTF) act through heterodimeric receptors comprised of LIF receptor and gp130 (14). We show in the current study that exogenous LIF stimulates terminal differentiation of Ob1771 and 3T3-F442A preadipose cells and induces adipocyte differentiation of multipotent mouse embryonic fibroblasts (MEF). Moreover, by using an antagonist of LIF receptor or genetically modified ES cells, we show that LIF receptor plays a key role during adipocyte differentiation. These results are at variance with previous reports showing an anti-adipogenic effect of LIF in 3T3-L1 preadipocytes (15, 16). The differential response to LIF of 3T3-L1 cells versus other cells is discussed.

The expression of C/EBPβ and C/EBPδ genes was rapidly induced in Ob1771 and 3T3-F442A preadipose cells after LIF addition. The role of the mitogen-activated protein kinase (MAPK) pathway in early events induced by LIF has been studied.

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been described (22, 23). The C/EBP (Fujix Bas 1000). Probes for LIF, ob, GAPDH, and LIF receptor have performed as described previously (21, 22). Quantification of the hybridization was conducted using a PhosphorImager apparatus (Fujix Bas 1000). Probes for LIF, ob, GAPDH, and LIF receptor have been described (22, 23). The C/EBPβ and C/EBPγ cDNAs were provided by S. L. McKnight (Tularik Inc., South San Francisco). The PPARγ cDNA was isolated from PPARγ2/SPORT plasmid and the a-FABP cDNA from pAL422 plasmid gift of P. Tontonoz and B.M. Spiegelman, Dana-Farber Cancer Institute, Boston.

RESULTS

The expression of LIF and LIF receptor genes was investigated during three defined stages of the program of 3T3-F442A adipocyte differentiation using a sensitive ribonuclease protection assay. Total RNAs were prepared from either exponentially growing cells corresponding to the adipoblast stage, from cells that reach confluence corresponding to the preadipose stage characterized at the molecular level by the early expression of specific genes such as A2COL6 (21), or from cells that express ob gene and accumulate triacylglycerol corresponding to the adipose stage of differentiation (22, 25). As shown in Fig. 1, levels of RNAs encoding the two forms of secreted LIF, i.e. the diffusible form (LIF-D) and the form associated to the extracellular matrix (LIF-M) (26), were higher in adipoblasts and preadipocytes than in mature adipocytes. In contrast, the LIF receptor was not detectably expressed in adipoblasts. Expression of LIF receptor was induced in preadipocytes in parallel to that of A2COL6 gene (not shown) and was maintained in mature adipocytes. Transcripts encoding the soluble form (sLIF-R) and the transmembrane form (tmLIF-R) of LIF receptor, which could be distinguished by RNase protection (23), showed a similar pattern of expression. The differential developmental expression of LIF and LIF receptor was found to be similar during adipocyte differentiation of the 3T3-F442A and Ob1771 clones (not shown). Expression of LIF and LIF receptor genes in mouse adipose tissue was investigated. The stromal-vascular fraction, containing adipoblasts and preadipocytes, and the adipocyte fraction, containing the bulk of mature adipocytes, were isolated from epididymal fat pads after cell dissociation by collagenase treatment. Total RNAs from each fraction were then prepared as described previously (21). LIF RNA was detected only in the stromal-vascular fraction, whereas LIF receptor was expressed in both fractions. These data are consistent with in vitro data and suggest that LIF and LIF receptor RNAs are expressed simultaneously in preadipocytes only.

The capacity of preadipocytes to secrete bioactive LIF was investigated. For that purpose we studied the capacity of Ob1771 preadipocytes to support pluripotent ES cells in coculture assays. It has been previously described that the maintenance of ES cells required the presence of LIF or LIF-related factors (24), and we have recently described the generation of convenient LacZ-marked ES cells in which β-galactosidase expression is restricted to pluripotent ES cells (19, 20). The indicator ES cells were plated on layers of confluent Ob1771 preadipocytes and maintained in either serum-supplemented or serum-free medium (17). Five days later, the number of β-galactosidase-positive colonies was determined. As shown in Fig. 2A, Ob1771 preadipocytes cells maintained in both culture condi-
were determined by quantification of β-galactosidase activity as described previously (59). Results are means (± S.D.) of colony numbers determined from duplicate wells. B, indicator ES cells were seeded and maintained for 5 days in 500 μl of standard medium (SM) or supplemented with 50 μl of medium conditioned (CM) by Ob1771 preadipocytes in the absence or presence of blocking anti-LIF antibody (αLIF). Maintenance of pluripotent ES cells were determined by quantification of β-galactosidase activity as described previously (59). Results are means ± S.E. from three independent experiments.

The effect of LIF on differentiation of Ob1771 preadipose cells was investigated in a chemically defined medium (17). Glycerol-phosphate dehydrogenase (GPDH) is a late marker of adipocyte differentiation that faithfully reflects cells containing triacylglycerol (17). Chronic exposure to LIF elicited a 20-fold increase in the specific activity of GPDH (Fig. 3) and the appearance of adipocyte-like cells (Fig. 4). The adipogenic effect of LIF was not unique to Ob1771 cells as a positive effect of LIF on differentiation of 3T3-F442A preadipocytes was observed (Fig. 3). LIF also induced adipocyte differentiation of BRL49653-treated multipotent MEF, indicating that the adipogenic effect of LIF is not restricted to clonal preadipose cell lines (Fig. 3). The effect of LIF on differentiation of 3T3-F442A preadipocytes was observed (Fig. 3). LIF also induced adipocyte differentiation of BRL49653-treated multipotent MEF, indicating that the adipogenic effect of LIF is not restricted to clonal preadipose cell lines (Fig. 3). The effect of LIF was then investigated as a function of exposure time. Exposure of Ob1771 cells to LIF from day 0 to 3 induced a 6-fold increase of GPDH activity, and exposure from day 0 to 7 was sufficient to achieve maximal response at day 11. In contrast, exposure after day 7 was ineffective (Fig. 3, lower panel). This observation suggests that LIF exerts its adipogenic effects by acting at an early step in the differentiation process, i.e. at the preadipose stage, which in turn dictates terminal events. Chronic exposure of Ob1771 cells to low concentration of the thiazolidinedione BRL49653 (20 nM), a PPARγ ligand (3, 29), promoted adipocyte differentiation to a low extent. However, LIF addition to the thiazo-
lidinedione-treated preadipose cells led to a dramatic increase in adipocyte differentiation (Fig. 3, lower panel and Fig. 4). Thus, LIF and PPARγ appear to stimulate differentiation in a synergic manner. Cross-talk between LIF signaling and PPARγ activation remains to be elucidated.

The LIF receptor antagonist hLIF05 was used to determine whether LIF receptor and LIF secreted by preadipocytes play a role during adipocyte differentiation. At the concentration of 0.5 μg/ml, hLIF05 had no toxic effect but inhibited adipocyte differentiation enhanced by 10 ng/ml LIF (not shown). As shown in Fig. 5, when hLIF05 was added during BRL49653-induced differentiation of Ob1771 cells in serum-free medium (no exogenous addition of LIF), the level of GPDH activity was reduced to 56 ± 7%. Another approach was used to examine the requirement of LIF receptor during the development of adipose cells as we have recently determined culture conditions that favor the commitment of ES cells into the adipocyte lineage (18). This model provides a valuable in vitro system for studying the role of genes active during adipocyte differentiation. The role of LIF and LIF receptor was addressed by investigating whether embryoid bodies derived from \( lifr^{-/-} \) ES cells (20) or \( lifr^{-/-} \) ES cells\(^2\) were able to undergo adipocyte differentiation. These mutant cells have been generated by gene targeting via two rounds of homologous recombination. LIF-null ES cells underwent adipogenesis with comparable efficiency to wild-type ES cells, as determined by the expression of adipocyte-specific genes. This result was in agreement with studies of LIF mutant mice, which indicated also that a lack of LIF expression did not prevent the development of adipose tissue (not shown). Likely, both in vitro and in vivo, LIF-related cytokines such as IL-6, CT-1, or CNTF could compensate for the lack of LIF. Therefore, the phenotype of LIF receptor-null ES cells was investigated. As previously reported (24), propagation of pluripotent ES cells can be maintained by addition of gp130-activating cytokines. Therefore, pluripotent \( lifr^{-/-} \) ES cells were maintained by addition of IL-6 together with soluble IL-6 receptor. These cells are responsive to IL-6/sIL-6R but are unresponsive to LIF, CT-1, or CNTF, which act through the LIF receptor-gp130 complex (20). The capacity of LIF receptor-deficient ES cells to undergo adipocyte differentiation was dramatically reduced. Only 5–7% of outgrowths derived from two independent clones of LIF receptor-deficient ES cells contained adipocyte colonies compared with 55–70% of outgrowths derived from wild-type ES cells. The \( lifr^{-/-} \) cells displayed an intermediate phenotype (Fig. 6A). The lower levels of expression of adipocyte-specific genes such as PPARγ and \( a-FABP \) in mutant cells compared with wild-type and heterozygotes were consistent with a suppression of terminal differentiation (Fig. 6B). Cardiomyocytes beating cells were detectable in wild-type and mutant cultures (not shown) and transcripts for myogenin, a skeletal muscle-specific marker, as well as for \( \alpha \) chain 2 of type VI collagen \((\alpha2COL6)\), a gene preferentially expressed by mesenchymal cells (30, 31), were present at similar levels in the three cell types (Fig. 5B). These observations indicate that the reduction of adipogenesis of LIF receptor-deficient ES cells did not reflect a general defect of these cells to undergo differentiation. Moreover, addition of IL-6 together with the soluble IL-6 receptor from day 7 to day 20 after embryoid formation, which corresponds to the permissive period for terminal differentiation (18), restored the capacity of LIF receptor-null cells to undergo adipocyte differentiation and to express adipocyte-specific genes (Fig. 6B, lane 4). These results indicate that LIF receptor plays a critical role in the process of terminal differentiation of preadipose cells into adipose cells derived from ES cells. Thus, detailed studies of LIF effects and the transducing machinery were undertaken.

The C/EBPβ and C/EBPδ transcription factors are expressed at the preadipose stage of the differentiation program. Although these trans-acting factors play a critical role in adipocyte differentiation, the nature of the extracellular factors that regulate their early expression is poorly known. Therefore, the regulation of C/EBP gene expression by LIF was investigated in Ob1771 and 3T3-F442A preadipose cells. As shown in Fig. 7, a dramatic increase in C/EBPδ expression in response to LIF was observed. This induction was rapid and transient as C/EBPδ transcript accumulation peaked within 1 h and decreased to basal level within 24 h. Similar kinetics were observed for C/EBPβ gene expression, although the stimulation remained lower due to a higher basal level at time 0. The accumulation of C/EBPβ and C/EBPδ RNAs by LIF was followed by an increase in C/EBP proteins. Accumulation of

\(^2\) M. Li and A. G. Smith, submitted for publication.
C/EBP proteins was detectable after 1 h, extended until 8 h, and disappeared 15 h after LIF addition (Fig. 7, lower panel). Not unexpectedly, the LIF receptor antagonist hLIF05 at 0.1 to 1 \( \mu \text{g/ml} \) abolished completely the stimulation of C/EBP\( \beta \) and C/EBP\( \delta \) by 10 ng/ml LIF but did not abolish that induced by 10 ng/ml IL-6 (not shown). This observation is consistent with the inhibition of adipogenesis by hLIF05 within the same range of concentrations (Fig. 5).

Addition of LIF to 3T3-F442A preadipose cells induced activation of p42 MAPK as determined by a decrease in electrophoretic mobility (Fig. 8). The level of activation was maximal after 10 min and returned to basal levels after 25 min. A similar response was observed for activation of p44 MAPK, whereas no activation of p38 MAPK and Jun kinase was detectable (not shown). Preincubation of preadipose cells with the specific MAPK kinase inhibitor PD98059 (32) prevented induction of C/EBP\( \beta \) and C/EBP\( \delta \) (Fig. 9A). This effect could not be mimicked by preincubation of cells with an inhibitor of protein synthesis such as cycloheximide (not shown). As shown in Fig. 9B, the inhibition of LIF-stimulated C/EBP gene expression was dose-dependent; 50% inhibition was reached at approximately 10 \( \mu \text{M} \) PD98059 in agreement with the IC\(_{50}\) value reported for this inhibitor (32). Requirement of p42/p44 MAPK activation for LIF-induced C/EBP gene expression was confirmed with the U0126 compound, which acts throughout a distinct mechanism to inhibit specifically MAPK kinase (33). A complete inhibition of LIF-induced C/EBP\( \delta \) expression was reached at 10 \( \mu \text{M} \) U0126 (not shown). As a 7-day exposure to LIF of preadipose cells in the presence of the PPAR\( \gamma \) ligand BRL49653 was sufficient to induce terminal differentiation of Ob1771 cells in serum-free conditions (Fig. 3) and as the MAPK kinase inhibitor PD98059 at 10 \( \mu \text{M} \) led to a significant effect on LIF induced-C/EBP gene expression (Fig. 9B), these conditions were used to evaluate the role of p42/p44 MAPK in terminal differentiation. No effect on cell viability of PD98059 alone was observed as determined by the lactate dehydrogenase activity (Fig. 9C). However, incubation of BRL49653- and LIF-treated cells with 10 \( \mu \text{M} \) PD98059 led to a decrease (60% \( \pm \) 10) of GPDH activity suggesting that p42/p44 MAPK activation was involved in terminal differentiation induced by addition of exogenous LIF. A similar result was obtained with 0.5 \( \mu \text{M} \) U0126.
Role of LIF Receptor in Adipocyte Differentiation

FIG. 9. Effect of MAPK kinase inhibitor PD98059 on LIF-stimulated C/EBP gene expression and on adipocyte differentiation induced by LIF. A, Ob1771 cells were maintained as in Fig. 7, and total RNAs were prepared from unstimulated cells (lane 1), or from stimulated cells with LIF for 30 min in the absence (lane 2), or in the presence of 50 μM PD98059 (lane 3) and analyzed for expression of indicated genes. B, total RNAs were prepared from Ob1771 stimulated cells with LIF for 1 h in the presence of the indicated concentrations of MAPK kinase inhibitor. Results are expressed by taking as 100% the signal obtained in the absence of inhibitor. The results are means ± S.E. from three independent experiments. C, confluent Ob1771 preadipose cells were maintained in serum-free conditions in the presence of 20 nM BRL49653 from day 0 to day 11. LIF (10 ng/ml) was added from day 0 to day 7 in the absence or in the presence of PD98059 (10 μM). In control, PD98059 (10 μM) was added on BRL-treated cells during the same period. In all conditions, GPDH activity (white bars), an index of cell viability, was observed. The results are means ± S.E. from three independent experiments. * signifies that the value of condition LIF plus PD98059 differs significantly from that of condition LIF; p < 0.01 assessed by Student’s t test.

instead of 10 μM PD98059 (not shown). Taken together, these results indicate that activation of p42/p44 MAPK plays a predominant role in mediating the early stimulation of C/EBPβ and C/EBPδ gene expression and adipocyte differentiation induced by LIF.

DISCUSSION

LIF and related cytokines, such as CTNF and CT-1 that act through LIF receptor-gp130 complexes, mediate an overlapping spectrum of biological activities. These cytokines are known to be involved in survival, proliferation, and differentiation of various cells, such as cells of the hematopoietic and osteogenic lineages (14). However, the role of the cytokines in the adipocyte program of differentiation has not previously been established. We show in our studies, by blocking LIF receptor activation or generating LIF receptor mutant cells, that the LIF receptor plays a critical role in the development of adipose cells in vitro. LIF receptor is activated by LIF and LIF-related cytokines. The fact that adipogenesis of LIF mutant ES cells is unchanged indicates that LIF alone is not critical in the program of adipocyte differentiation. Experiments using an antagonist of LIF receptor or a specific anti-LIF blocking antibody and undifferentiated ES cells as an indicator of the presence of gp130-activating cytokines revealed that, in addition of LIF, a LIF-related cytokine appears secreted by Ob1771 preadipocytes. This cytokine which might well be CTNF or CT-1, which act like LIF through the LIF receptor-gp130 complexes, could compensate for the lack of LIF. These observations indicate that several cytokines acting through LIF receptor-gp130 signaling are secreted at the preadipose stage of the differentiation program. Besides the adipose tissue, studies of the regulation of expression of these cytokines could give some insights into the role of preadipocytes present in bone marrow (34) and skeletal muscle (35).

Homoygous LIF receptor mutant mice are not viable (36, 37) precluding the study of the role of this signaling pathway during the development of white adipose tissue which takes place postnatally. Therefore, we have used genetically modified ES cells combined with conditions of culture to commit stem cells into the adipogenic pathway to provide evidence that LIF receptor plays a role in the development of adipose cells in vitro. Two phases have been identified in the development of adipogenesis from ES cells. A first phase, which corresponds to a permissive period for the commitment of ES cells into the adipocyte lineage, and a second phase, which corresponds to the permissive period for terminal differentiation (18). The fact that addition of IL-6/sIL-6R only during the permissive period for terminal differentiation of ES cells restored the adipocyte phenotype strongly suggests that gp130 signaling pathway is not involved in the commitment of mesenchymal stem cells toward the adipogenic pathway but plays a role in the terminal differentiation of preadipocytes into adipocytes. The similar levels of expression of myogenin and A2COL6 in outgrowths derived from LIF receptor-mutant ES cells and in those derived from wild-type ES cells is in agreement with this hypothesis.

Our results strongly suggest an autocrine/paracrine mechanism by which LIF receptor activation induces early events that are critical for subsequent differentiation. This proposal is based upon three observations as follows: (i) LIF is secreted by preadipocytes that express also LIF receptor; (ii) blocking LIF receptor activation decreases differentiation enhanced by BRL49653; and (iii) a short term exposure to LIF at the preadipose stage is sufficient to trigger adipose cell differentiation. LIF stimulates expression of adipogenic transcription factor C/EBPβ and C/EBPδ and acts in a synergistic manner with PPARγ ligand to induce adipocyte differentiation of Ob1771 preadipose cells. This differentiating effect of LIF is not confined to Ob1771 preadipose cells as treatment of 3T3-F442A preadipose cells and mouse embryonic fibroblasts with LIF together with a specific PPARγ ligand led to the rapid activation of C/EBP gene expression and subsequently to adipocyte differentiation. Ectopic expression of C/EBPs in the presence of PPARγ activator leading to the conversion of fibroblastic cells into mature adipocytes has been previously reported (8, 9).

The adipogenic effects of LIF reported here are at variance with previous reports of an anti-adipogenic effect of this cytokine when added to the adipogenic mixture required to induce
differentiation of 3T3-L1 (15, 16) and bone marrow-derived BMS2 clonal lines (38). There are several possible mechanisms to explain the opposite effect of LIF on differentiation of 3T3-L1 cells compared with 3T3-F442A and Ob1771 cells as well as to mouse embryonic fibroblasts. First, it has been shown with bone cells that LIF is able to induce opposite effects on cells of the same lineage depending on their stage of development (39). Second, depending upon the factors present, LIF could act in a synergistic manner and stimulate cells to proliferate, which has been shown to antagonize adipocyte differentiation (40). LIF displayed no mitogenic activity in Ob1771 and 3T3-F442A preadipose cells in serum-supplemented medium. In contrast, we observed that LIF induced a potent mitogenic activity in 3T3-L1 cells accompanied by an inhibition of differentiation (not shown). The inhibitory effect of LIF on adipogenesis of 3T3-L1 cells was decreased in serum-free conditions suggesting that unidentified serum factors could also have an anti-adipogenic effect in addition to that of LIF. These observations emphasize the importance to study the LIF signaling pathway under conditions where the additional effects of unidentified serum factors can be minimized.

It has been demonstrated in various cell types that signal transducers and activators of transcription (STAT) pathway may also be triggered from LIF receptor (14). STAT3 plays a central role in LIF-induced self-renewal and maintenance of undifferentiated ES cells (41, 42), differentiation of myeloid leukemic M1 cells (43), and differentiation of neuroepithelial precursors into astrocytes (44). LIF is a potent inducer of STAT3 in 3T3-F442A and Ob1771 preadipocytes and in 3T3-L1 adipocytes (45). However, a role of STAT3 in adipocyte differentiation has not yet been reported. Experiments are in progress in our laboratory to investigate the role of STAT3 activation in LIF-induced early events in adipogenesis. Regarding the role of MAP kinase pathway in adipocyte differentiation, it has been shown that p38 MAPK is required for adipogenesis, in part through its effect on C/EBPβ activity (46). The nature of the effectors triggering p38 MAPK activation during adipocyte differentiation has not yet been reported, but LIF can be excluded as no stimulation of this activity is observed under conditions where C/EBPβ and -δ expression is up-regulated (not shown). When the experiments described in this paper were ongoing, Stephens and colleagues published a report (45) showing that LIF activates p42/p44 MAPK pathway in 3T3-L1 adipocytes, but no target genes were identified. We show herein that activation of p42/p44 MAPK is required for stimulation of C/EBP expression and terminal differentiation of preadipocytes into adipocytes induced by LIF. Our results demonstrate for the first time that the p42/p44 MAP kinase pathway can mediate a differentiation response to LIF. These results are in agreement with the fact that depletion of p42/p44 MAPK using an antisense oligonucleotide strategy blocked the ability of preadipocytes to undergo differentiation in response to insulin (47). In contrast, Font de Mora et al. (48) have reported that inhibition of MAPK activation by PD98059 for a short time (2 h) has little effect on differentiation of 3T3-L1 cells. Moreover, adipocyte differentiation is inhibited in 3T3-L1 transfectant cells overexpressing hyperactive MAP kinases in a constitutive manner. This may be a reflection of the functional output of MAPK which depends on the extent and the duration of activation. It has been shown in CCL39 fibroblasts that the mitogenic activity of various growth factors is only associated with a prolonged stimulation of MAP kinases (49), and it has been reported in PC12 cells that the same factor could trigger cell proliferation or differentiation depending on the duration and extent of activation of p42/p44 MAPK (50, 51). Therefore, it is possible that constitutive activation of MAPK pathway in 3T3-L1 transfectants promotes extensive cell proliferation that antagonizes adipocyte differentiation. Inclusion of a MAPK kinase inhibitor would attenuate overproliferation and would favor differentiation. Altogether, these results reflect that the MAPK pathway regulates a fine balance between cell growth and differentiation (52, 53).

An alternative mechanism favoring either positive or negative modulation of MAPK pathway on adipocyte differentiation is the phosphorylation of PPARγ. Interestingly, phosphorylation of PPARγ by MAP kinases can lead to opposite effects. On the one hand, phosphorylation of PPARγ by MAP kinases activated by mitogens leads to inhibition of adipogenesis and direct phosphorylation of PPARγ by MAPK activated by anti-adipogenic factors has been shown (54, 55). On the other hand, phosphorylation of PPARγ by MAP kinases activated by an adipogenic factor, i.e. insulin, enhanced adipogenesis although direct phosphorylation of PPARγ by MAPK remains in this case to be demonstrated (56). The observation that activation of MAP kinase pathway by different stimuli leads to opposite effects on adipocyte differentiation has also been previously reported for differentiation of PC12 cells (57). Detailed comparison of p42/p44 MAPK activation triggered by LIF and anti-adipogenic factors in preadipose cells, including the extent and duration of the activation as well as nuclear translocation events (58–60), should bring further insights into the regulatory role played by the MAP kinase pathway in adipogenesis.

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