Galectin-12 Is Required for Adipogenic Signaling and Adipocyte Differentiation*

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Galectin-12 is a member of the galectin family consisting of β-galactoside-binding proteins with conserved carbohydrate recognition domains. This protein is preferentially expressed in peripheral blood leukocytes and adipocytes. We previously showed that galectin-12 is induced by cell cycle block at the G0 phase and causes G1 arrest when overexpressed (Yang, R.-Y., Hsu, D. K., Yu, L., Ni, J., and Liu, F.-T. (2001) J. Biol. Chem. 276, 20252–20260). Here, we show that the galectin-12 gene is expressed in mouse preadipocytes and is up-regulated when preadipocytes undergo cell cycle arrest, concomitant with acquisition of the competence to undergo differentiation in response to adipogenic hormone stimulation. Following a brief down-regulation 1 day after adipogenic treatment, its expression was once again markedly elevated when cells underwent terminal differentiation. Down-regulation of endogenous galectin-12 expression by RNA interference greatly reduced the expression of the adipogenic transcription factors CCAAT/enhancer-binding protein-β and -α and peroxisome proliferator-activated receptor-γ and severely suppressed adipocyte differentiation as a result of defective adipogenic signaling. We conclude that galectin-12 is required for signal transduction that conveys hormone stimulation to the induction of adipogenic factors essential for adipocyte differentiation. The findings suggest that galectin-12 is a major regulator of adipose tissue development.

Adipose tissue is a major site of energy storage and expenditure. It also actively regulates food uptake and whole body metabolism by secreting a number of hormones and cytokines (1–3). However, excessively increased adipose tissue mass leads to obesity, which is a major risk factor for non-insulin-dependent diabetes mellitus (type II diabetes) and hypertension. It is also linked to some types of cancers and immune dysfunctions. Adipose tissue mass is determined by both the size and number of fat cells, or adipocytes. Increase in fat cell number is accomplished by the differentiation of preadipocytes to mature adipocytes, or adipogenesis (4).

Much has been learned about the transcriptional regulation of adipocyte differentiation in the past 20 years (5, 6) due to the availability of in vitro models of adipogenesis based on preadipocyte cell lines such as 3T3-L1 and 3T3-F442A (7, 8). The major transcription factors in the transcription network of adipogenesis include CCAAT/enhancer-binding proteins, which belong to the basic leucine zipper class of transcription factors (9), and peroxisome proliferator-activated receptor-γ (PPARγ),1 a member of the nuclear hormone receptor superfamily (10). Confluent 3T3-L1 preadipocytes can be differentiated into white adipocytes by treatment with a combination of an agent that elevates intracellular cAMP levels, a glucocorticoid, and insulin, commonly abbreviated as MDI for isobutylmethylxanthine, dexamethasone, and insulin. CCAAT/enhancer-binding protein (C/EBP)-β and C/EBPα are the first transcription factors induced after MDI treatment, which can be detected within 2 h. After a long lag of ~2 days, they become competent to activate the expression of C/EBPα and PPARγ (11), which positively regulate each other’s expression and which mediate the transcription of a number of downstream genes that characterize the adipocyte phenotype (12–14).

Galectins are a family of animal lectins with conserved carbohydrate recognition domains (CRDs) for β-galactoside (15). They are present in most species of the animal kingdom, including lower organisms such as nematodes and higher organisms such as mammals. In mammals, 14 members have been identified to date (16). Some of the members, especially galectin-3 and galectin-1, have been extensively studied, and experimental results suggest that these lectins may have diverse functions, including regulation of pre-mRNA splicing, cell proliferation and differentiation, and apoptosis (17–23). Most galectins have wide tissue distribution. For example, galectin-3 is abundantly present in the epithelia of several organs (24) as well as in various inflammatory cells, including monocytes/macrophages (25). Consistent with the lack of a classical signal sequence, galectins are mainly intracellular proteins (26). However, a number of studies have demonstrated the secretion of these proteins (27). Consistent with their spatial distribution, these proteins appear to function both intracellularly and extracellularly. The extracellular functions are likely to be due to carbohydrate binding properties and, in many cases, are inhibited by specific free carbohydrate, whereas the intracellular functions may not be related to carbohydrate binding (28). Although all galectins contain at least one homologous CRD in their sequence, different members exhibit different localization and expression patterns, suggesting distinct functions for each member of the family (21, 28, 29).

1 The abbreviations used are: PPARγ, peroxisome proliferator-activated receptor-γ; MDI, adipogenic hormone mixture consisting of isobutylmethylxanthine, dexamethasone, and insulin; C/EBP, CCAAT/enhancer-binding protein; CRD, carbohydrate recognition domain; IGF-1, insulin-like growth factor-1; CREB, cAMP response element-binding protein; ERK, extracellular signal-regulated kinase; IRS-1, insulin receptor substrate-1; siRNA, small interfering RNA.
We (31) and others (30) have previously cloned human galectin-12 cDNA and found that it encodes a galectin with two CRDs. The N-terminal CRD is highly homologous to those of other galectins, whereas the C-terminal CRD shows significant divergence from the consensus sequence (31). Its mRNA contains AU-rich motifs in the 3′-untranslated region, and the start codon of the major open reading frame lies in a sequence context suboptimal for translation initiation (31), suggesting vigorous post-transcriptional regulation at the level of mRNA stability (32) and translation efficiency (33). The expression of this gene is very restricted, with high level expression only in adipocytes (30) and peripheral blood leukocytes (31). Galectin-12 is up-regulated when cells are blocked at the G1 phase, and ectopic expression of this protein causes cell cycle arrest at the G1 phase with concomitant cell growth suppression (31). Its expression in adipocytes is up-regulated by insulin-sensitizing siRNA duplexes with 3′/H11032 overhangs on each strand targeting the distinct sequences in mouse galectin-12 mRNA: 5′-AACGUACGCG-3′ (Sigma) (MDI) in 2T cells were subsequently cultured in medium with insulin only. Cell cycle distribution during adipocyte differentiation was determined by flow cytometry of propidium iodide-stained cells fixed with ethanol as described previously (31).

Lipid droplets in adipocytes were stained with the lipophilic dye oil red O (Sigma) (44, 45). Cells were fixed for 1 h in 3.7% formaldehyde, rinsed in 80% isopropyl alcohol, and stained with 1.8 mg/ml oil red O in 60% isopropyl alcohol and 0.4% dextrin for 15 min. Excessive oil red O stain was then washed away with three changes of water (30 s each), with the last one containing 1:1000 SYBR Green I (Molecular Probes, Inc.) to stain the nuclei. Stained cells were visualized under a Zeiss inverted microscope equipped with argon (488 nm) and helium/neon (543 nm) lasers using a 100×/1.40 NA objective, a rhodamine filter set and a rhodamine filter set for SYBR Green I and oil red O, respectively. Images were acquired and analyzed with Zeiss LSM 510 software. Quantification of oil red O staining was achieved by extracting lipid-associated dye with 100% isopropyl alcohol for 15 min before spectrophotometry to measure the absorbance at 510 nm (46). Results were then normalized to protein contents of the samples.

**Analysis of Gene Expression—** RNA extraction and reverse transcription-PCR were performed as described (31) with the following primers in the PCR steps after reverse transcription: 5′-CCGTCTACGGTCTCTCCGCG-3′ and 5′-TTGGACCCCTTCTTAAGTGG-3′ for mouse galectin-12, 5′-CATTCCCTCTGGATACGCGACGTAAGAGTG-3′ and 5′-TCAGATCTCTGCTACCTTACCGAAGTTGG-3′ for mouse adipsin, 5′-CTGAGTCTCTGCTATCCATTTCTGATG-3′ for mouse adipsin, 5′-CTGAGTCTCTGCTATCCATTTCTGATG-3′ for mouse adipsin, 5′-CTGAGTCTCTGCTATCCATTTCTGATG-3′ for mouse adipsin, 5′-CTGAGTCTCTGCTATCCATTTCTGATG-3′ for mouse adipsin, 5′-CTGAGTCTCTGCTATCCATTTCTGATG-3′ for mouse adipsin, and 5′-TTGGACCCCTTCTTAAGTGG-3′ for mouse galectin-12. Primers 5′-TGA-AGTCGCTTGGATACGCGACGTAAGAGTG-3′ and 5′-CATGTAGCCATGAGTCACCAAG-3′ were used for mouse glyceroldehyde-3-phosphate dehydrogenase as a control. For Western blotting, cells were lysed in SDS-sample buffer and boiled for 5 min, and the lysates were resolved on SDS-polyacrylamide gels, transferred to Immobilon-P membrane (Millipore Corp.), and probed with the indicated antibodies as described (31).

**Analysis of Adipogenic Signal Transduction—** Cells were lysed at different time points of MDI stimulation in SDS sample buffer; boiled for 5 min, and subjected to Western blotting with Western blotting and 5′-TTGGACCCCTTCTTAAGTGG-3′ antibodies against phosphotyrosine (PY20), the insulin receptor β-subunit, and insulin receptor substrate-1 (IRS-1) were from BD Biosciences. Rabbit anti-galectin-12 antibody was generated by immunizing rabbits with inclusion bodies of the C-terminal CRD of galectin-12 produced with expression vector pET-14b (Novagen) and Echerichia coli strain BL21-SI (Invitrogen). For affinity purification of the antibody, the C-terminal CRD of galectin-12 was expressed as a bacterial Nasa fusion protein with vector pET-43.1 (Novagen). The fusion protein was purified with Talon affinity beads (Clontech) and conjugated to Sepharose 4B. These Sepharose affinity beads were then used to purify antibodies specific for galectin-12 from the antisera by affinity chromatography.

**Analysis of Galectin-12 during Adipocyte Differentiation—** The mouse preadipocyte cell line 3T3-L1 is a continuous sub-strain of 3T3 (Swiss albino) developed through clonal isolation (8). These cells become competent to undergo a preadipose-to-adipose conversion as they progress from a rapidly dividing to a confluent and contact-inhibited state and are frequently used in an in vitro model of adipocyte differentiation that closely recapitulates the in vivo process (4). To determine the timing of galectin-12 expression in adipocyte differentiation, galectin-12 mRNA levels were determined by reverse transcription-PCR (Fig. 1A) during the differentiation of 3T3-L1 cells. Consistent with our previous observation that galectin-12 arrests the cell cycle at the G1 phase (31), we found that its expression was low

**RESULTS**

Expression of Galectin-12 during Adipocyte Differentiation— The mouse preadipocyte cell line 3T3-L1 is a continuous sub-strain of 3T3 (Swiss albino) developed through clonal isolation (8). These cells become competent to undergo a preadipose-to-adipose conversion as they progress from a rapidly dividing to a confluent and contact-inhibited state and are frequently used in an in vitro model of adipocyte differentiation that closely recapitulates the in vivo process (4). To determine the timing of galectin-12 expression in adipocyte differentiation, galectin-12 mRNA levels were determined by reverse transcription-PCR (Fig. 1A) during the differentiation of 3T3-L1 cells. Consistent with our previous observation that galectin-12 arrests the cell cycle at the G1 phase (31), we found that its expression was low.
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Galectin-12 Is Required for Adipocyte Differentiation—The correlation of galectin-12 expression with adipocyte differentiation suggests an important function of this protein in adipogenesis. We employed RNA interference to study the role of galectin-12 in adipocyte differentiation by knocking down the expression of endogenous galectin-12 with siRNAs specific for galectin-12 mRNA. It has been shown that siRNA with 2-nucleotide overhangs efficiently and specifically suppresses gene expression (42, 49, 50). Two control siRNAs for luciferase and galectin-3, respectively, and three siRNAs targeting distinct sequences in galectin-12 mRNA were used in our experiments. When 3T3-L1 cells were treated with siRNAs before being subjected to the pro-differentiative regimen, all three galectin-12 siRNAs significantly down-regulated galectin-12 expression, whereas the two control siRNAs did not affect the expression (Fig. 2a). After stimulation with adipogenic hormones, control siRNA-transfected cells accumulated large quantities of lipid droplets, a telltale sign of adipocyte differentiation. In contrast, cells transfected with galectin-12 siRNAs contained many fewer lipid droplets (Fig. 2b). Quantitation of oil red O staining indicated that cells transfected with galectin-12 siRNAs accumulated only one-third as much triglyceride seen in control cells (Fig. 2c), indicating a major defect in adipogenesis.

Aside from serving as a depot for triglyceride, another important function for adipose tissue is to regulate glucose homeostasis in response to insulin stimulation. Insulin sensitivity is acquired during adipocyte differentiation as the insulin receptortor and its substrates are up-regulated by C/EBPα (12). Western blotting revealed high levels of insulin receptor expression in control cells 7 days after adipogenic treatments. In contrast, no insulin receptor expression was detectable after the same treatment of cells transfected with galectin-12 siRNAs (Fig. 2d). These cells also expressed less IRS-1 compared with control cells (Fig. 2d).

Impaired Induction of Critical Adipogenic Transcription Factors and Adipocyte Differentiation Markers in Galectin-12-deficient Cells—Based on their temporal sequence of expression as well as gain- and loss-of-function studies both in vitro and in vivo, it is now well recognized that C/EBPβ and C/EBPδ are rapidly induced in response to adipogenic stimulation. They then activate the expression of two major adipogenic transcription factors, C/EBPa and PPARγ, which cross-regulate each other’s expression (6). PPARγ-activated gene expression is responsible for most of the mature adipocyte phenotype (13), except insulin sensitivity, which is ascribed to the C/EBPa-induced expression of insulin receptor and IRS-1 genes (12). Because adipogenic hormone-induced adipocyte differentiation and insulin receptor and IRS-1 expression are impaired in galectin-12-deficient cells (Fig. 2), we tested whether galectin-12 is required for the induction of these critical transcription factors. In control cells, adipogenic hormone treatment induced C/EBPβ expression in 2 h. C/EBPβ protein levels in these cells continued to rise and peaked after 1 day of treatment (Fig. 3a). In comparison, C/EBPβ induction in cells transfected with galectin-12 siRNA was dramatically impaired, and its protein levels in these cells remained low during 2 days of adipogenic hormone treatment (Fig. 3a). Consistent with this, these cells also contained greatly reduced levels of C/EBPa and PPARγ, the two downstream targets of C/EBPβ, during the 6 days of stimulation with adipogenic hormones (Fig. 3b).

Adipin (also called complement factor D, C3 convertase activator, and properdin factor D) is a serine protease secreted by mature adipocytes and is considered as a late marker of adipocyte differentiation (51). During the 6 days of adipogenic stimulation, adipin expression was markedly suppressed in cells transfected with galectin-12 siRNA (Fig. 3c). The expression of the adipocyte fatty acid-binding protein aP2, an early adipocyte differentiation marker, was also reduced by galectin-12 knockdown, although to a lesser extent (Fig. 3c).

Defective Adipogenic Signaling in Galectin-12 Knockdown Cells—Failure to induce C/EBPβ in galectin-12-deficient cells suggests a defect in upstream signaling. We therefore set out to determine the effect of galectin-12 knockdown on the proximal adipogenic signal transduction events (Fig. 4). Adipogenic hormone stimulation leads to the activation of insulin/IGF receptors on preadipocytes and elevated intracellular cAMP levels. This results in the phosphorylation and activation of Akt/protein kinase B, a major regulator of adipogenesis (52, 53), and the transcription factor CREB, which is believed to mediate the expression of C/EBPβ (54). Western blotting with phosphospecific antibodies revealed that both the basal and hormone-stimulated phosphorylation of Akt were dramatically reduced in galectin-12-deficient cells (Fig. 4). Western blotting with phosphospecific antibodies revealed that both the basal and hormone-stimulated phosphorylation of Akt were dramatically reduced in galectin-12-deficient cells (Fig. 4). ERK phosphorylation was also defective. Moreover, we found that both the magnitude and duration of CREB phosphorylation were greatly reduced (Fig. 4), which explains the impaired induction of C/EBPβ in galectin-12 knockout cells (Fig. 3e). The IGF-1 receptor is the predominant receptor for insulin to generate adipogenic signals in preadipocytes (55). It initiates downstream signaling by autophosphorylation of tyrosine residues. By immunoprecipitation with anti-phosphotyrosine antibody and Western blotting of the precipitates with anti-IGF-1 receptor antibody, we...
found that tyrosine phosphorylation of the IGF-1 receptor itself was not affected by galectin-12 deficiency (Fig. 4).

**DISCUSSION**

Mouse 3T3-L1 preadipocytes express galectin-12 at low levels. The changes in galectin-12 expression correlate with a number of important events during adipocyte differentiation (Fig. 1). First, galectin-12 is up-regulated at both the mRNA and protein levels as cells enter contact inhibition-induced growth arrest. This coincides with the acquisition of competence by the preadipocytes to initiate the whole differentiation program in response to adipogenic stimulation (4–6). The brief down-regulation of galectin-12 1 day after MDI addition correlates with the maximal expression of C/EBPβ and mitotic clonal expansion (11, 48). It is already known that C/EBPβ is required for mitotic clonal expansion (56), which is essential for adipogenesis (48). This suggests that, although up-regulated expression of galectin-12 at the time of MDI addition is important for adipogenic signaling, as will be discussed below, brief down-regulation of galectin-12 after C/EBPβ induction may be required for the optimal expression and/or activity of this transcription factor. Low level galectin-12 expression during this period may allow C/EBPβ to transactivate the C/EBPα and PPARγ genes, after which the galectin-12 gene is greatly elevated, probably as a result of transactivation by the latter two transcription factors. The expression of galectin-12 during adipogenesis also correlates with the cell cycle status. Galectin-12 mRNA and protein levels are high in cells in cell cycle arrest as a result of contact inhibition or terminal differentiation and low in proliferating subconfluent cells and cells at the phase of mitotic clonal expansion (Fig. 1). This is consistent with our previous observations that this gene is up-regulated when cells are blocked at G1 or the G1/S boundary of the cell cycle and that its ectopic expression causes G1 arrest (31).

Suppression of adipocyte differentiation by RNA interference of galectin-12 expression (Fig. 2) lends strong support to the hypothesis that this protein is a major regulator of adipogenesis. Triglyceride storage and insulin-regulated lipid and glucose metabolism are the major functions of adipocytes. They accumulate fat at times of energy excess and mobilize it when free energy is needed. These processes are all regulated by insulin-initiated signal transduction (57, 58), and Akt is a key enzyme involved in this process (59). Defects in the formation of lipid droplets and the expression of the insulin receptor and IRS-1 in galectin-12 knockdown cells (Fig. 2) indicate that this protein regulates nearly all important aspects of adipocyte differentiation.

The transcription network controlling adipocyte differentiation is already well understood, with several proteins of the C/EBP family and the nuclear hormone receptor PPARγ playing central roles in this process (6). Among them, C/EBPβ and C/EBPδ are the first to be induced. They then transactivate genes for C/EBPα and PPARγ, which cross-regulate each other’s expression (12). PPARγ can correct many defects in adipogenesis of C/EBPα-deficient fibroblasts with the exception of insulin sensitivity (12, 60). C/EBPα-deficient adipocytes show reduced gene expression of the insulin receptor and IRS-1 and a complete absence of insulin-stimulated glucose transport (12). C/EBPα is therefore critical for the establishment of insulin sensitivity in adipocytes. Our results show that the whole adipogenic transcription network is compromised when endogenous galectin-12 is suppressed (Fig. 3). Failure of the induction of C/EBPβ gene (Fig. 3a) should account for the greatly reduced expression of C/EBPα and PPARγ in these cells (Fig,
the two key adipogenic factors downstream of C/EBPα. Experiments were repeated four times with receptor antibody. Similar results were obtained with other control and antibody PY20 and subsequent Western blotting with anti-IGF-1 antibodies. Tyrosine phosphorylation of the IGF-1 receptor (Fig. 3a) was determined by immunoprecipitation of tyrosine-phosphorylated proteins (10 μg) was loaded per lane. The expression of the late (adipin) and early (aP2) adipocyte differentiation markers and the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was also monitored throughout the period of differentiation by reverse transcription-PCR with specific primers (c). Similar results were obtained with other control and galectin-12 siRNAs. Results are representative of three separate experiments.

Fig. 3. Expression of adipogenic transcription factors and adipocyte differentiation markers is defective in galectin-12 knockdown cells. (a) Three days after transfection with the indicated siRNAs (time 0), the pro-adipogenic hormone mixture was added for various lengths of time, and protein was extracted for Western blotting to detect C/EBPβ induction. The expression of C/EBPα and PPARγ, the two key adipogenic factors downstream of C/EBPβ, was determined throughout the period of differentiation by Western blotting with the respective antibodies (b). Note the presence of two major translation products for each of these transcription factors as the result of the use of alternative translation initiation sites (69, 70). An equal amount of total protein (10 μg) was loaded per lane. The expression of the late (adipin) and early (aP2) adipocyte differentiation markers and the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was also monitored throughout the period of differentiation by reverse transcription-PCR with specific primers (c). Similar results were obtained with other control and galectin-12 siRNAs. Results are representative of three separate experiments.

Fig. 4. Galectin-12 is required for adipogenic signal transduction. Three days after transfection with the indicated siRNAs, 3T3-L1 cells were treated with the adipogenic hormone mixture for various lengths of time before being harvested for Western blotting with antibodies to ERK, Akt, CREB, or their respective phosphorylated (p) forms. Tyrosine phosphorylation of the IGF-1 receptor (p-IGF1R) was determined by immunoprecipitation of tyrosine-phosphorylated proteins with antibody PY20 and subsequent Western blotting with anti-IGF-1 receptor antibody. Similar results were obtained with other control and galectin-12 siRNAs. Experiments were repeated four times with similar results.

3b). Suboptimal expression of the latter two transcription factors is not permissive for adipocyte differentiation, as reflected by the impaired triglyceride accumulation (Fig. 2, b and c) and the defective expression of the insulin receptor and IRS-1 (Fig. 2d) and adipocyte differentiation markers (Fig. 3c) in these cells.

We further demonstrated that galectin-12 is indispensable for signal transduction upstream of the adipogenic transcription network (Fig. 4). The most striking defect in adipogenic signaling of galectin-12 knockdown cells is Akt phosphorylation. It is known that Akt activation is critical for adipocyte differentiation (55). Inhibition of phosphatidylinositol 3-kinase, a kinase upstream of Akt, suppresses adipogenesis (61), whereas the expression of constitutively active Akt induces adipocyte differentiation (52). Mice deficient in Akt display impaired PPARγ expression and adipogenesis (53). Akt and protein kinase A may also phosphorylate CREB (62–64), whereas ERK contributes to CREB-directed transcription, probably through recruitment of coactivators (54). Dominant-negative CREB inhibits hormone-induced adipocyte differentiation when expressed in preadipocytes, and ectopic expression of constitutively active CREB promotes adipogenesis (65). Activation of CREB may be directly responsible for the induction of C/EBPβ, whose promoter region contains functional CAMP response element sites and is activated by CREB (54, 65, 66). Defective CREB activation in galectin-12-deficient cells is therefore likely to account for the impeded induction of C/EBPβ.

This is the first galectin to be shown to modulate the differentiation of a key cell type of metabolism by regulating the signaling pathway instrumental to energy storage and expenditure (57). Our results indicate that galectin-12 regulates the early steps of adipogenesis. It may participate in the establishment of a competent state in growth-arrested preadipocytes, enabling them to respond to hormone stimulation and to undergo adipocyte differentiation. This is consistent with the early up-regulation of galectin-12 during this process, when cells become G1-arrested and responsive to adipogenic stimulation, before the induction of the transcription network (Fig. 1). In this regard, galectin-12 may be a factor in the commitment of the adipocyte lineage: its expression levels may determine how a cell responds to a certain environment cue, either differentiation into adipocytes or another lineage or self-renewal (proliferation). The fact that tyrosine phosphorylation of the IGF-1 receptor is not reduced and that activation of downstream kinases is defective (Fig. 4) suggests that galectin-12 acts downstream of IGF-1/insulin receptors and upstream of ERK and Akt. This probably occurs at the level of the adaptor proteins Shc and IRS, in particular their recruitment and phosphorylation by the receptors. The exact nature of the involvement of galectin-12 in adipogenic signaling is not known. It was previously reported that galectin-12 localizes in the cytoplasm in a speckled pattern as well as in the nucleus (30). Although its presence in the nucleus (where the central cell cycle machinery is located) is consistent with its function in cell cycle regulation, cytoplasmic distribution is in agreement with an additional role for galectin-12 in the regulation of proximal signaling events. Galectin-12 may directly modulate these signaling events through physical interactions with relevant components of the signaling pathway. In fact, galectins have been shown to interact with other proteins independent of binding to carbohydrates, although these interactions are often lactose-inhibitable and involve their CRDs (22, 67, 68). The clear divergence of the C-terminal domain of galectin-12 from traditional galectin CRDs (31) makes it more likely that this domain is not utilized for binding to lactose, but to ligands of another nature instead. In any case, identification of intracellular ligands will be critical to the elucidation of the exact role of galectin-12 in signal transduction.
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Our data indicate that the initial up-regulation of galectin-12 (when cells undergo growth arrest) is required for the response of preadipocytes to adipogenic hormone stimulation. In view of its anti-proliferative activity (31), transient down-regulation of galectin-12 during the first day of hormone treatment may be essential for mitotic clonal expansion to occur. Subsequent up-regulation of galectin-12 expression after clonal expansion coincides with C/EBPα and PPARγ expression and could be important for the establishment and maintenance of insulin sensitivity in mature adipocytes. Consistent with this, insulin-sensitizing PPARγ agonists up-regulate galectin-12 expression (30), whereas agents that cause insulin resistance in adipocytes down-regulate its expression (34). Galectin-12 could therefore be an effective drug target against obesity-associated diseases such as diabetes.

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