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Lecture

A pathogenic mechanism leading to partial lipodistrophy and prospects for pharmacological treatment of insulin resistance syndrome

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Abstract. The understanding of a common complex phenotype such as insulin resistance can be favoured by evaluation of monogenic syndromes. Clinical definition, pathogenesis, and therapeutical strategies for the insulin resistance syndrome can thus be improved by the characterization at the molecular genetic level of monogenic forms of lipodystrophies. Here we report experimental evidence on the pathogenic mechanism underlying insulin resistance in a rare form of laminopathy, due to mutation of the *LMNA* gene coding for lamin A/C, the Dunning-type familial partial lipodystrophy (FPLD). The defect, consisting in the intranuclear accumulation of mutant unprocessed precursors of lamin A, reduces the amount of the DNA-bound adipocyte transcription factor sterol regulatory element binding protein 1 (SREBP1) and lowers the peroxisome proliferator-activated receptor (PPARγ) expression, causing the impairment of pre-adipocyte differentiation. The treatment with the PPARγ ligand troglitazone (TDZ) is able to rescue the adipogenic program. Since FPLD recapitulates the essential metabolic abnormalities of the common insulin resistance syndrome, the beneficial effects of TDZ on monogenic lipodystrophies might provide a clue as to the future treatment strategies also for the common syndrome of insulin resistance. (www.actabiomedica.it)

Key words: Lipodystrophy, insulin resistance, lamin A/C, chromatin, SREBP1, PPARγ, adipocyte, LMNA gene, laminopathies, FPLD, pathogenic mechanisms, drug treatment

Introduction

Insulin resistance syndrome or metabolic syndrome is a disorder frequently occurring in individuals with android obesity associated with a cluster of metabolic abnormalities, including glucose intolerance, dyslipidemia, hypertension, which result into premature atherosclerosis, even before the onset of diabetes (1). At the molecular genetic level it has been possible to characterize several lipodystrophies, whose disease phenotype mainly consists into an anomalous distribution of the body fat or a generalized loss of adipose tissue (2). In fact, lipodystrophic patients exhibit loss of adipose tissue stores in defined sites, with accumulation of fat in other ones, as well as in liver and muscle. The majority of lipodistrophyes have been characterized as monogenic forms of insuline resistance, due to mutations of genes coding for either the nuclear lamin A/C, such as Dunnigan-type familial partial lipodystrophy (FPLD) (3), mandibular acral dysplasia (MADA) (4), syndromes of partial lipodistrophy with cardiomyopathy (5), Hutchinson-Gilford progeria (HGPS) (6), atypical Werner's syndrome (WS) (7), or the peroxisome proliferator-activated receptor (PPARγ), such as Dunnigan-like familial partial lipodystrophy (8), or seipin, such as Berar-

dinelli-Seip congenital generalized lipodystrophy (BSCL) (9). Furthermore, lipodystrophy syndrome may be induced by an highly active anti-retroviral treatment (HAART) (10).

The mechanisms underlying the insulin resistance are presently unclear; their definition might help to design preventive or treatment starategies. A possible approach to the pathogenic mechanisms leading to insulin resistance syndrome, is to address attention to a genetically extreme form, such as the monogenic form of insulin resistance described as FPLD.

Molecular genetic bases for familial partial lipodystrophy

FPLD phenotype, not present at birth but during puberty, is characterized by absence of subcutaneous fat from extremities, resulting in well-defined musculature, whilst intramuscular and bone marrow fat is preserved; on the other hand, excess fat deposition occurs at neck, face, back, and intra-abdominally (11). Although FPLD phenotype is more obvious in women, men are equally affected. The metabolic markers of FPLD include insulin resistance, resulting in diabetes with aging, dyslipidemia and hypertension; menstrual abnormalities, hirsutism, acanthosis nigricans are often present.

The mutations found in *LMNA* gene, resulting in FPLD, are clustered only in exons 8 and 11, encoding the globular C-terminal domain of type-A lamins. The most frequent FPLD-linked *LMNA* mutation results in a substitution of arginine at position 482 with a neutral aminoacid. The FPLD-linked *LMNA* missense mutations result in characteristic phenotypic alterations in a large proportion of nuclei, observed in cultured skin fibroblasts from affected subjects; these consist in abnormal shape, indented or herniated profile and increased fragility (12, 13). Furthermore, intranuclear aggregates, mostly localized close to the nuclear lamina, were found in R482L mutated fibroblasts (14), which have been demonstrated to be constituted by accumulation of pre-lamin A sequestering the adipocyte transcription factor sterol regulatory element binding protein 1 (SREBP1) (15). Interestingly, the proportion of altered nuclei increased with the patient age and with the severity of the clinical phenotype.

These findings opened a very intriguing question on the pathogenic role of specific *LMNA* mutations in FPLD as well as in other laminopathies with a lipodystropic phenotype. In fact, the mutational hotspot found in FPLD-linked phenotype suggests that a specific alteration in a lamin A/C region involved in the binding with transcription factors involved in adipocyte differentiation/function, could result typical lipodystrophic alterations. On the other hand, other laminopathies, including MADA, HGPS and WS, which are due to mutations not restricted to the hotspot region found in FPLD, present lipodystrophic alterations together with insulin resistance, resulting in premature aging.

Here we report experimental evidence suggesting that altered pre-lamin A processing is a common mechanism leading to lipodystrophy linked to mutations of *LMNA* gene. Furthermore we demonstrate that the resulting pre-lamin A accumulation reduces the rate of DNA-bound SREBP1 and lowers PPARγ espression, causing impairment of pre-adipocyte differentiation. This defect, which could be considered the pathogenic mechanism of *LMNA*-linked lipodystrophy, can be rescued by treatment with troglitazone, a known PPARγ ligand activating the adipogenic program.

FPLD cells carryng a R482L lamin A/C mutation show dysmorphic nuclei and lamin A/C intranuclear aggregates

Fibroblast cultures were established from skin biopsy explants obtained from FPLD patient carrying an R482L lamin A/C mutation, as well as from unaffected controls or Emery-Dreifuss (EDMD2) patients. In 15-20% of FPLD cells nuclei appeared enlarged and with an irregular profile with respect to control. By immunolabeling with anti-lamin A/C antibody a reduction of the labeling at the nuclear rim was observed in these dysmorphic FPLD nuclei; the same antibody revealed intranuclear lamin A/C aggregates (10 to 30 per nucleus) distributed throughout the nucleoplasm, but mostly localized close to the nu-

clear lamina (14). Interestingly, by double immunofluorescence labeling, emerin, which in control nuclei co-localizes with lamin A/C at the nuclear rim, was not found at the lamin A/C nuclear aggregates detected in FPLD cells. Since it has been demonstrated that lamin A/C binds emerin and the strength of the binding mainly involve lamin A for retaining emerin at the nuclear envelope (16), we evaluated the emerin-lamin A interaction in FPLD cell lysates by immunoprecipitation. The immunoblot of the immunoprecipitated complex showed that lamin A failed to coprecipitate with emerin in FPLD cells. This suggests that the R482L mutation, occurring in the tail domain that includes the sequence that binds emerin, could either impair lamin A-emerin binding, or lamin A interaction with a third molecule necessary to stabilize the complex. In any case, the impairement of the emerin-lamin A binding reported in this study (14), represents the first evidence of altered in vivo protein-protein interaction in FPLD.

Mutated lamin A/C accumulating in FPLD cells affects trascriptional activity

Accumulation of mutant lamin A/C due to the lack of normal functional interactions with nuclear partners does not only result into a dysmorphic nuclear phenotype, but in the impairement of nuclear activities. Since it has been reported that cells transfected with a mutated lamin A show a reduced RNA polymerase II-mediated transcription (17), transcriptional activity has been evaluated in R482L FPLD cells, incorporating bromouridine (BrU) into transcribed RNA. A strong reduction of incorporated BrU was found just in FPLD fibroblast bearing abnormal lamin A/C aggregates, demonstrating that the R482L mutation interferes with RNA polymerase II-mediated transcription, whilst it does not affect nucleolar RNA synthesis (14). This finding provided the first experimental evidence thet RNA transcription is affected in a laminopathy, as a consequence of lamin A/C to correctly assemble at the nuclear lamina, forming abnormal intranuclear aggregates.

In the ligth of these findings, the pathogenic hypothesis previosly speculated by several authors (18-21), that laminopathies may occur owing to alteration in gene expression as a consequence of lamin A/C improper localization, is gaining experimental evidence. However, more than one mechanism is likely to be involved in the pathogenesis of laminopathies and, as far as laminopathies with lipodystrophy is concerned, a crucial role could be played by nuclear factors involved in adipocyte differentiation, that have been found to interact with nuclear envelope proteins, including lamin A/C (22).

Aggregates accumulating in laminopathic cells, including FPLD, are constituted by pre-lamin A

In recent times, a further mechanism has been proposed as a key pathogenic factor in a large group of laminopathies. Altered pattern of heterochromatin distribution has been found in EDMD2 (23), FPLD (15), MADA (24) and HGPS (13). It is likely that mutations affecting lamin A result in defective interactions with chromatin-associated proteins, including HP1, which mediates the association of heterochromatin with the nuclear envelope (25), thus impairing the localization of heterochromatin at the nuclear periphery. Other proteins that exert a transcriptional control could also be involved: A-type lamins, indeed, provide scaffolds for proteins like pRb, GCL, the transcriptional repressor MOK2, BAF and chromatin remodeling complexes (21). DNA-binding domains are present in the rod domain of lamin A/C (26); however, *LMNA* mutations found in laminopathies result into a normal or slightly reduced expression of lamin A/C. On the other hand, some mutations result in impressive accumulation of pre-lamin A at intranuclear sites (13,15).

The precursor of lamin A has a CAAX motif at the C-terminus and undergoes post-translational modifications including farnesylation, methylation and proteolytic cleavages; these modifications significantly increase the hydrophobicity of the C-terminus of lamin A (27). We recently provided evidence on the mechanism that links the accumulation of unprocessed lamin A aggregates in the nucleus and chromatin rearrangement. The fibroblasts from MADA patients show a dramatic loss of heterochromatin in nuclei ac-

cumulating pre-lamin A, whilst the proteins HP1 and H3K9 become partly soluble by Triton X-100, suggesting that heterochromatin is partly unstructured (24). In HGPS fibroblasts we found analogous defects, which are not related to a loss of mature lamin A, but to accumulation of progerin, a truncated farnesylated/methylated pre-lamin A (28).

Accumulation of pre-lamin A is not restricted to FPLD, but occurs also in MAD and WS cells; also these laminopathies, however, are characterized by lipodystrophic disorders that are not present in other laminopathies, such as EDMD2, where pre-lamin A amount is not increased. In any case, why lipodystrophy-linked mutations can affect lamin A processing is matter of speculation. However, a farnesylated pre-lamin A is accumulated, as suggested by both the use of farnesyltransferase inhibitors (15), and by the demonstration that mutations of the zinc metalloproteinase ZPMSTE 24, leading to accumulation of farnesyl-pre-lamin A due to impaired cleavage of the farnesylated protein, cause MADA in humans (29).

A specific mechanism leading to FPLD involves interactions between pre-lamin A and SREBP1

In FPLD, accumulation of pre-lamin A and chromatin defects have been recently reported (15), and the demonstration of interaction of pre-lamin A with an adipocyte-specific transcription factor appears to account for a pathogenic mechanism leading to lipodystrophy (Fig. 1).

In fact, *in vivo* binding of pre-lamin A to the adipocyte transcription factor SREBP1 has been demon-

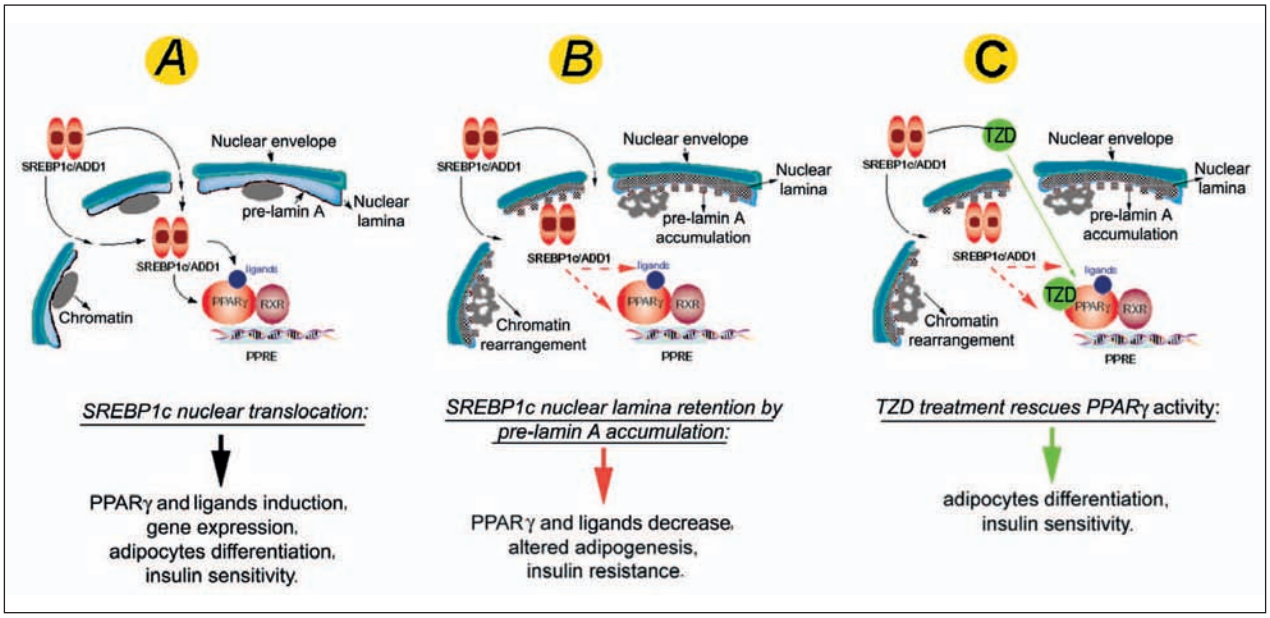

Figure 1. Possible mechanism by which mutant lamin A expression can result into FPLD lipodystrophic phenotype and molecular targets of TZD. **A**. In normal conditions the transcriptional control of adipogenesis is mediated by the SREBP1c/ADD1 complex that, upon translocation to the nuclear interior, activates the expression of PPARγ and the production of an endogenous PPARγ ligand (arrows). The PPARγ/retinoic acid X receptor (RXR) dimer targets the peroxisome proliferator response element (PPRE) to regulate the expression of genes involved in adipocyte differentiation and insulin sensitivity. **B**. Phenotypic alterations of FPLD cells include abnormal nuclear profiles, rearrangements of the peripheral heterochromatin, and accumulation of unprocessed mutant lamin A at the nuclear rim. Because SREBP1 translocation into the nucleus requires interactions with the nuclear envelope, the presence of abnormal accumulation of pre-lamin A, could alter the delivery of the transcription factor (dashed arrows). This, in turn, might impair the PPARγ-dependent adipocyte differentiation and reduce insulin sensitivity. **C**. Pre-adipocytes accumulating pre-lamin A (by farnesyltransferase inhibitors or by transfection with uncleavable pre-lamin A mutant) present a reduced expression of PPARγ and fail to differentiate into adipocytes. TZD treatment rescue adipogenic differentiation, because, even in the presence of SREBP1 sequestration, it can act as coactivator of the PPARγ-dependent gene pathway

strated to occur in FPLD cells accumulating pre-lamin A at the nuclear rim, reducing the pool of DNA-bound active transcription factor (15). The retention of SREBP1 at the nuclear rim, described in pre-adipocytes treated with Indinavir, that causes lipodystrophy as side-effect, is associated with increased levels of pre-lamin A and reduced PPARγ expression (10). This suggests that the retention of SREBP1 at the nuclear periphery is due to the accumulation of pre-lamin A, irrespective of the occurrence of *LMNA* mutations, as in acquired lipodystrophies due to drugs that, causing pre-lamin A accumulation, impair adipocyte differentiation (30). Adipocyte differentiation, indeed, requires the triggering by SREBP1 of PPARγ (1). Accumulation of lamin A precursors by farnesyl transferase inhibitors or by overexpression of uncleavable pre-lamin A, on the other hand, causes down-regulation of PPARγ expression (15). Therefore, PPARγ induction, a key step of adipocyte differentiation, is altered in lipodystrophic laminopathies, as

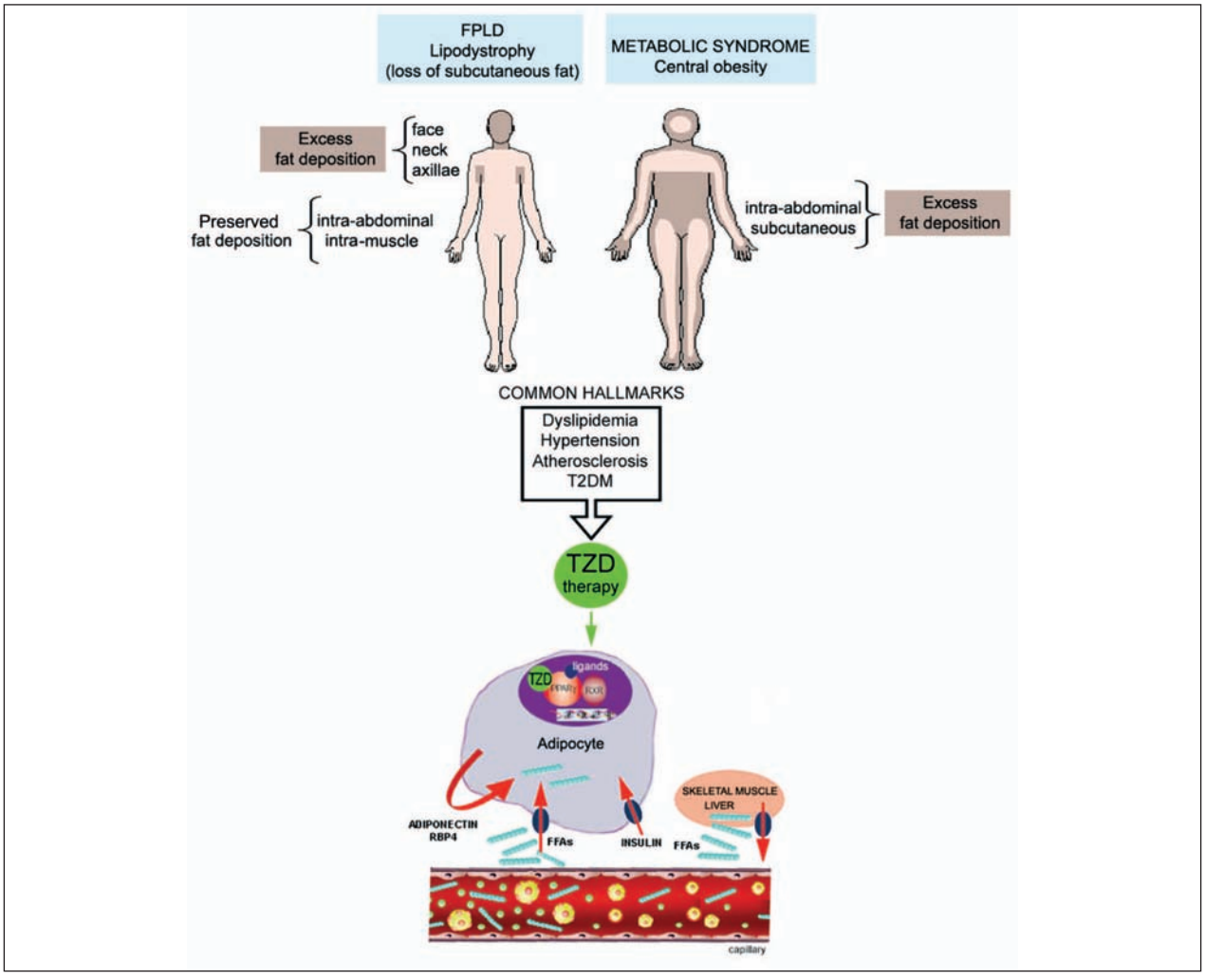

Figure 2. Possible targets of TZD therapy in inherited and acquired lipodystrophies, characterized by specific phenotypic alterations of the adipose tissue but sharing metabolic hallmarks. TZD effects on adipocytes can occur at different levels: i) as reported in Fig. 1 the rescue of adipogenic differentiation can occur by activation of the PPRE-regulated gene expression through a binding of TZD that acts as a coactivator of PPARγ; ii) enhancement of free fatty acids (FFA) trapping by adipocytes, sequestering them away from insulin-sensitive tissues such as skeletal muscles and liver; iii) increased expression of adipocytokines with autocrine and paracrine activity, such as adiponectin and the retinol-binding protein 4 (RBP4), that mediate the insulin-sensitizing effects of TDZ

proposed for acquired lipodystropies (10).

PPARγ expression is regulated depending on the body district (31), suggesting that pre-lamin A accumulation may elicit different effects at given body regions depending on the extent of the transcription factor activation required in that area. This could account for the selective involvement of some but not all fat depots in partial lipodystrophies.

Search of a common mechanism leading to rare monogenic diseases, type 2 diabetes mellitus (T2DM), and obesity

In previous sessions we reported experimental evidence on the possible pathogenic mechanism leading to loss of adipose tissue stores in some anatomical sites in a particular class of inherited lipodystrophies, due to mutation of the *LMNA* gene, including FPLD, MAD and HGPS. These genetic lipodystrophies may represent interesting models for acquired or drug-induced lipodystrophies, such as those induced by HAART, being the mechanism of accumulation of pre-lamin A demonstrated to occur in both cases. In addition, the genetic dystrophies are of additional interest because they share impaired metabolic features with highly common disorders, including type 2 diabetes mellitus and obesity (Fig. 2). In fact, FPLD showed metabolic changes that are similar to those found in the common metabolic syndrome, including, besides insulin resistance, raised plasma triglycerides, free fatty acids and C-reactive protein(CPR), with reduced high-density lipoprotein (HDL) cholesterol, serum leptin and adiponectin (2). FPLD women were also at high risk for early atherosclerosis (1). It is interesting to note that the main concern with most of lipodystrophies either genetic, aquired or drug-induced, is the high prevalence of cardiac (atherosclerosis) and hepatic complications (NASH), associated to signs of premature aging, conceivably due to altered fat repartition and insulin resistance (32). Moreover, the recent finding of a novel *LMNA* heterozygous missense mutation in lamin A in a female patient presenting a type A syndrome of insulin resistance without clinical lipodystrophy suggests that primary alterations in insulin signalling could also occur in laminopathies (33). Interestingly, the mechanism could involve dysregulation of PKCα which both binds lamin A (34) and inhibits insulin signalling (35).

The development of insulin resistance, with its attendant clinical and metabolic complications, including atherosclerosis and premature aging, found in FPLD patients could yield new insights that could apply to common insulin resistance. Insulin resistance in human syndromes of lipodystrophy is considered to be due to an insufficient adipose tissue capacity to buffer dietary fatty acids (FAs), with consequent lipotoxicity, due to deposition of triglycerides (TG) and acyl-CoA in insulin-sensitive tissues, which can be increased by leptin deficiency. Expression of PPARγ plays a key role not only in adipocyte differentiation, but also in the entraining of adipose tissue metabolism to nutritional state, by upregulating genes that mediate FAs uptake and trapping (36); furthermore, its high expression in macrophages, which infiltrate the dysfunctional adipose tissue of obese subjects (37), may be pathophysiologically crucial. In any case, clinical insights into PPARγ function can be achieved by thiazolidinedione (TZD) therapy, a class of insulin-sensitizing drugs that selectively acts on PPARγ (38). Insulin sensitization by TDZ through PPARγ activation could be interpreted as a consequence of the ability of PPARγ to expand depot-selective adipose tissue, including subcutaneous adipose tissue, with concomitant reduction in visceral depots (38), stressing the functional metabolic differences between abdominal and femoro-gluteal subcutaneous fat (39). TDZs are likely to act on adipose tissue to enhance its ability to act as a pump for dietary FAs, sequestering them in adipocytes and removing them from other insulin-sensitive tissues, including skeletal muscle (40). In fact, although the level of PPARγ is low in skeletal muscle, the muscle total mass and the presence of 70% of insulin-mediated glucose disposal, suggest that PPARγ may play important physiological effects in insulin-stimulated glucose uptake in muscle. Furthermore, TDZ treatment reduces nonhalcoholic hepatic steatosis in human (41), suggesting that TDZ plays beneficial effects on FAs trapping in adipose tissue that counteract hepatic lipid accumulation.

Early onset hypertension is another feature found in both genetic lipodystrophies, including FPLD and HGPS, and in acquired metabolic lipodystrophies (42). It must be considered that PPARγ is expressed in vascular smooth muscle and endothelial cells; transgenic mice expressing dominant-negative P465L PPARγ were found to be hypertensive (43). Furthermore, PPARγ dysfunction in macrophages might contribute to accelerated atherogenesis (37).

Targets for a pharmacological treatment of lipodystrophyies

PPARγ has been identified as a member of the nuclear receptor family just over a decade ago. Although some aspects of the role of this molecule in human metabolic disease have not been completely clarified, the knowledge of its biological role suggested its utility a s therapeutic target. Recently, it has been hypothesized that PPARγ pathway may be a potential target of TZDs for intervention in osteoporosis (44), because PPARγ haploinsufficiency has been shown to promote osteogenesis through enhanced osteoblast formation (45).

We recently demonstrated the possibility of rescuing adipogenic differentiation of pre-adipocytes by reducing pre-lamin A accumulation through PPARγ agonists (15).

In pre-adipocytes PPARγ is transcribed following SREBP1 activation (10); when pre-lamin A accumulation was induced by farnesyltransferase inhibitors (mevinolin or FTI-277) or by transfecting cells with an uncleavable pre-lamin A mutant, the PPARγ expression was strongly downregulated. Interestingly, PPARγ expression was also reduced in cells accumulating pre-lamin A following drug treatment, as shown to occur in FPLD cells. Accumulation of pre-lamin A and downregulation of PPARγ expression greatly reduced adipocyte differentiation of pre-adipocytes, as revealed by oil red O staining. The treatment of pre-adipocytes accumulating pre-lamin A with the PPARγ ligand TZD rescued the differentiation process into mature adipocytes, suggesting that the adipogenic program can be rescued reducing PPARγ dysfunction and/or mislocalization due to accumulating pre-lamin A (15).

Conclusions

The experimental evidence here reported support the assumption that the evaluation of pathogenic mechanisms of monogenic human lipodystropy syndromes might provide valuable insights to understand insulin resistance occurring in common acquired disorders, including T2DM and obesity. Furthermore, some new acquisitions have been obtained on the possible mechanisms of action of the thiazolidinedione therapy that can rescue both adipogenic differentiation and metabolic pathways in adipocytes.

Figure 1 schematically reports the possible mechanism by which TZD can rescue adipogenic differentiation in pre-adipocytes which, as in FPLD, are induced to accumulate pre-lamin A by farnesyltransferase inhibitors or by transfection with an uncleavable pre-lamin A mutant. In these situations, the adipogenic differentiation program is impaired by a reduced amount of SREBP1 translocated to the nuclear interior, owing to an altered interaction with mutant lamin A at the nuclear envelope level. However, TZD is able to overcome this block, by acting as a coactivator of PPARγ which controls the peroxisome proliferation response element (PPRE) gene expression pathway leading to adipocyte differentiation. On the other hand, as other nuclear hormone receptors, PPARγ is a ligand-activated transcription factor, and TZDs, which bind to PPARγ with reasonably high affinity, can act as a coactivator of the adipogenic pathway (46).

In Figure 2 are reported some possible sites of action of TDZs on adipose tissue in humans. Insulin sensitization by PPARγ activation upon TDZ administration could result into depot-selective responses of adipose tissue, resulting in selective accumulation of subcutaneous adipose tissue, with reduction of visceral depots (31). Furthermore, TDZs can enhance the capacity of adipose tissue to trap free FAs, sequestering them away from other insulin-sensitive tissues such as liver and skeletal muscles (40). Finally, insulin sensitivity might be influenced by the release of specific adipocytokines by adipose tissue cells, such as adiponectin and the retinol-binding protein 4 (42).

These findings give an important insight into possible therapeutic approaches to lipodistrophy by

TZD, which have been already reported to elicit some promising effects (47).

Because FPLD recapitulates the essential metabolic abnormalities seen in common insulin resistance syndrome (2), the beneficial effect of TZDs in lipodistrophies might provide a clue as to future treatment strategies also for the common syndrome of insulin resistance.

References

1. Hegele RA. Molecular basis of partial lipodystrophy and prospects for therapy. *Trends Mol Med* 2001; 7: 121-6.
2. Hegele RA. Monogenic forms of insulin resistance: apertures that expose the common metabolic syndrome. *Trends Endocrinol Metab* 2003; 14: 371-7.
3. Shackleton S, Lloyd DJ, Jackson, et al. LMNA, encoding lamin A/C, is mutated in partial lipodystrophy. *Nat Genet* 2000; 24: 153-6.
4. Novelli G, Muchir A, Sangiuolo F, et al. Mandibuloacral dysplasia is caused by mutation il LMNA-encoding lamin A/C. *Am J Hum Genet* 2002; 71: 426-31.
5. Bhayana S, Siu VM, Joubert GI, Clarson CL, Cao H, Hegele RA. Cardiomyopathy in congenital complete lipodystrophy. *Clin Genet* 2002; 61: 283-7.
6. De Sandre-Giovannoli A, Bernard R, Cau P, et al. Lamin A truncation in Hutchinson-Gilford progeria. *Science* 2003; 300: 2055.
7. Chen L, Lee L, Kudlow BA et al. LMNA mutations in atypical Werner's syndrome. *Lancet* 2003; 362: 440-5.
8. Hegele RA, Cao H, Frankowski C, Mathews ST, Leff T. PPARG F388L, a transactivation-deficient mutant, in familial partial lipodystrophy. *Diabetes* 2002; 51: 3586-90.
9. Magre JJ, Delepine M, Khallouf E, et al. Identification of the gene altered in Berardinelli-Seip congenital lipodystrophy on chromosome 11q13. *Nat Genet* 2001; 28: 365-370.
10. Caron M, Auclar M, Sterlingot H, Kornprobst M, Capeau J. Some HIV protease inhibitors alter lamin A/C maturation and stability, SREBP-1 nuclear localization and adipocyte differentiation. *AIDS* 2003; 17: 2437-2444.
11. Garg A, Peshock RM, Fleckenstein JL. Adipose tissue distribution pattern in patients with familial partial lipodystrophy (Dunnigan variety). *J Clin Endocrinol Metab* 1999; 84: 170-4.
12. Vigouroux C, Auclair M, Dubosclard M, et al. Nuclear envelope disorganization in fibroblasts from lipodystrophic patients with heterozygous R482Q/W mutations in the lamin A/C gene. *J Cell Sci* 2001; 114: 4459-68.
13. Goldman RD, Shumaker DK, Erdos MR, et al. Accumulation of mutant lamin A causes progressive changes in nuclear architecture in Hutchinson-Gilford progeria syndrome. *Proc Natl Acad Sci USA* 2004; 101: 8963-8.
14. Capanni C, Cenni V, Mattioli E, et al. Failure of lamin A/C to functionally assemble in R482L mutated familial partial lipodystrophy fibroblasts: altered intermolecular interaction with emerin and implications for gene transcription. *Exp Cell Res* 2003; 291: 122-34.
15. Capanni C, Mattioli E, Columbaro M, et al. Altered prelamin A processing is a common mechanism leading to lipodystrophy. *Hum Mol Genet* 2005; 14: 1489-502.
16. Gruenbaum Y, Wilson KL, Harel A, Goldberg M, Cohen M. Nuclear lamins-structural proteins with fundamental functions. *J Struct Biol* 2000; 129: 313-23.
17. Spann TS, Goldman AE, Wang C, Huang S, Goldman RD. Alteration of nuclear lamin organization inhibits RNA polymerase II-dependent transcription. *J Cell Biol* 2002; 156: 603-8.
18. Wilson KL. The nuclear envelope, muscular dystrophy and gene expression. *Trends Cell Biol* 10: 125-9.
19. Burke B, Stewart CL. Life at the edge: the nuclear envelope and human disease. *Nature Rev Mol Cell Biol* 2002; 3: 575-85.
20. Maraldi NM, Lattanzi G, Sabatelli P, Ognibene A, Squarzoni S. Functional domains of the nucleus: implications for Emery-Dreifuss muscular dystrophy. *Neuromusc Disorders* 2002; 12: 815-23.
21. Maraldi NM, Lattanzi G. Linkage of lamins to fidelity of gene transcription. *Crit Rev Euk Gene Exp* 2005; 15: 277-93.
22. Lloyd DJ, Trembath RC, Shackleton S. A novel interaction between lamin A and SREBP1: implications for partial lipodystrophy and other laminopathies. *Hum Mol Genet* 2002; 11: 769-77.
23. Sabatelli P, Lattanzi G, Ognibene A, et al. Nuclear alterations in autosomal-dominant Emery-Dreifuss muscular dystrophy. *Muscle Nerve* 2001; 24: 826-9.
24. Filesi I, Gullotta F, Lattanzi G, et al. Alterations of nuclear envelope and chromattin organization in mandibuloacral dysplasia, a rare form of laminopathy. *Physiol Genomics* 2005; 23: 150-8.
25. Kourmouli N, Theodoropoulos PA, Dialynas G, et al. Dynamic associations of heterochromatin protein 1 with the nuclear envelope. *EMBO J* 2000; 19: 6558-68.
26. Stirlé V, Couprie J, Ostlund C, et al. The carboxyl-terminal region common to lamin A and C contains a DNA binding domain. *Biochemistry* 2003; 42: 4819-28.
27. Maske CP, Hollinshead MS, Higbee NC, Bergo MO, Young SG, Vaux DJ. A carboxyl-terminal interaction of lamin B1 is dependent on the CAAX endoprotease Rce1 and carboxylmethylation. *J Cell Bio* 2003; 162: 1223-32.
28. Columbaro M, Capanni C, Mattioli E, et al. Rescue of heterochromaatin organization in Hutchinson-Gilford progeria by drug treatment. *Cell Mol Life Sci* 2005; 62: 2669-78.
29. Agarwal AK, Fryns JP, Auchus RJ, Garg A. Zinc metalloproteinase, ZMPSTE24, is mutated in mandibuloacral dysplasia. *Hum Mol Genet* 2003; 12: 1995-2001.
30. Solomon CS, Leitner JW, Goalstone ML. Dominant negative alpha-subunit of farnesyl- and geranyl-transferase I

inhibits insulin-induced differentiation of 3T3-L1 pre-adipocytes. *Int J Obes Relat Metab Disord* 2003; 27: 40-7.
31. Sewter CP, Blows F, Vidal-Puig A, O'Rahilly S. Regional differences in the response of human pre-adipocytes to PPARgamma and RXR-alpha agonists. *Diabetes* 2002; 51: 718-23.
32. Capeau J, Magrè J, Lascols O, et al. Diseases of adipose tissue: genetic and acquired lipodystrophies. *Bioch Soc Trans* 2005; 33: 1073-1077.
33. Young J, Morbois-Trabut L, Couzinet B, et al. Type A insulin resistance syndrome revealing a novel lamin A mutation. *Diabetes* 2005; 54: 1873-8.
34. Martelli AM, Bortul R, Tabellini G, et al. Molecular characterization of protein kinase C-alpha binding to lamin A. *J Cell Biochem* 2002; 86: 320-30.
35. Zick Y: Role of Ser/Thr kinases in the uncoupling of insulin signaling. *Int J Obes Relat Metab Disord* 2003; 27: S56-S60.
36. Tontonoz P, Hu E, Devine J, Beale EG, Spiegelman BM. PPAR gamma 2 regulates adipose expression of the phosphoenolpyruvate carboxykinase gene. *Mol Cell Biol* 1995; 15: 351-7.
37. Weisberg SP, McCann D, Desai M, Rosenmaum M, Leibel RL, Ferrante AW. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* 2003; 112: 1796-808.
38. Larsen TM, Toubro S, Astrup A. PPARgamma agonists in the treatment of type II diabetes: is increased fatness commensurate with long-term efficacy? I*nt J Obes Relat Metab Disor* 2003; 27: 147-61.
39. Garg A: Regional adiposity and insulin resistance. *J Clin Endocrinol Metab* 89: 42066-4210.
40. Ye JM, Dzamko N, Cleasby ME, et al. Direct demonstration of lipid sequestration as a mechanism by which rosiglitazone prevents fatty-acid-induced insulin resistance in the rat: comparison with metformin. *Diabetologia* 2004; 47: 1306-13.
41. Promrat K, Lutchman G, Uwaifo G, et al. A pilot study of pioglitazone treatment for nonalcoholic steatohepatitis. *Hepatology* 2004; 39: 188-96.
42. Semple RK, Chatterjee VKK, O'Rahilly S. PPARγ and human metabolic disease. *J Clin Invest* 2006; *116*: 581-9.
43. Tsai YS, Kim HJ, Takahashi N, et al. Hypertension and abnormal fat distribution but not insulin resistance in mice with P465L PPARγ. *J Clin Invest* 2004; 114: 240-9.
44. Pei L, Tontonoz P. Fat's loss is bone's gain. *J Clin Invest* 2004; 113: 805-6.
45. Akune T, Ohba S, Kamekura S, et al. PPARγ insufficiency enhances osteogenesis through osteoblast formation from bone marrow progenitors. *J Clin Invest* 2004; 113: 846-55.
46. Rosen ED, Walkey CJ, Puigserver P, Spegelman BM. Transcriptional regulation of adipogenesis. *Genes Develop* 2000; 14: 1293-307.
47. Owen KR, Donohoe M, Ellard S, Hattersley AT. Response to treatment with rosiglitazone in familial partial lipodystrophy due to mutation in the LMNA gene. *Diabet Med* 2003; 20: 823-7.

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