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Guardian of corpulence: a hypothesis on p53 signaling in the fat cell

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Abstract

Adipocytes provide an organism with fuel in times of caloric deficit, and are an important type of endocrine cell in the maintenance of metabolic homeostasis. In addition, as a lipid-sink, adipocytes serve an equally important role in the protection of organs from the damaging effects of ectopic lipid deposition. For the organism, it is of vital importance to maintain adipocyte viability, yet the fat depot is a demanding extracellular environment with high levels of interstitial free fatty acids and associated lipotoxic effects. These surroundings are less than beneficial for the overall health of any resident cell, adipocyte and preadipocyte alike. In this review, we discuss the process of adipogenesis and the potential involvement of the p53 tumor-suppressor protein in alleviating some of the cellular stress experienced by these cells. In particular, we discuss p53-mediated mechanisms that prevent damage caused by reactive oxygen species and the effects of lipotoxicity. We also suggest the potential for two p53 target genes, START domain-containing protein 4 (*StARD4*) and oxysterol-binding protein (*OSBP*), with the concomitant synthesis of the signaling molecule oxysterol, to participate in adipogenesis.

Keywords

adipogenesis; diabetes; lipotoxicity; oxysterol; p53

The adipocyte is a truly remarkable cell in many aspects. In recent years several studies have identified adipose tissue as an important endocrine organ involved in regulating whole-body energy homeostasis through the actions of a range of adipocyte-specific secreted cytokines, commonly referred to as ‘adipokines’ [1–3]. The tight connection between adipocytes and several types of cancer further highlights the significance of this endocrine role [4–6]. In addition, the adipocyte stores energy in the form of triglycerides to supply the organism with free fatty acids as fuel in times of fasting. In the evolution of animals, the advent of the adipocyte must have been a life-transforming event, liberating animals to roam unconstrained by the need for a direct and continuous supply of energy [7,8]. As an added bonus, with the development of the adipocyte as a lipid-sink, these cells were further enabled to protect other organs from

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the deleterious effects of excessive levels of cholesterol, triglycerides and free fatty acids. These effects (commonly referred to as 'lipotoxicity') include the induction of endoplasmic reticular (ER)-stress by excessive cholesterol loads and general oxidative damage due to increases in reactive oxygen species (ROS) [9–13]. Failure of the fat depot to store lipids and cholesterol properly results in lipotoxicity that contributes to several serious morbidities associated with insulin-resistance, such as cardiovascular disease, kidney disease and diabetes [14].

The importance of the adipocyte in ensuring proper lipid homeostasis is further highlighted by the onset of devastating dyslipidemia and insulin resistance in both pharmacologically induced and genetically afflicted lipodystrophic patients [15,16]. A milder example is provided in the elderly, where loss of adipogenic differentiation potential with aging is associated with ectopic fat storage [17]. Conversely, the flip side also holds true. Adipocytes from the adipocyte-specific insulin-receptor knockout mouse can still maintain lipid homeostasis, but are no longer able to take up and store glucose in response to insulin; yet, blood glucose levels in these mice are normal, the mice are protected from age- and diet-induced obesity and, interestingly, they live significantly longer [18,19]. The protection against diet-induced obesity could be explained by lack of the permissive effect of insulin on triglyceride storage in fat or by lack of the antilipolytic effects of insulin in adipocytes, causing the organism to burn fat rather than store it. These data elegantly illustrate the deleterious effects fat has on longevity and health [18,19].

Building a fat cell

The differentiation of a committed preadipocyte into an adipocyte is mediated by several profound morphological changes in the cell, such as the formation of caveolae in the plasma membrane, the formation of a unilocular lipid droplet, and a vesicular system regulating the uptake of glucose in response to insulin. Caveolae are omega-shaped membrane invaginations of 50–150 nm in diameter under structural control of the caveolin proteins [20,21]. These lipid-raft-derived structures occupy 30% of the surface of the adipocyte plasma membrane and play an important role in the regulation of lipid and cholesterol uptake by the adipocyte. The concomitant induction of lipogenic and lipid-handling genes, such as the adipocyte-specific fatty acid transporter aP2 and lipoprotein lipase (LPL), provides the adipocyte with its ability to handle and store huge quantities of lipids in its lipid droplet [22]. This defining trait of the adipocyte, the large central lipid droplet, is derived from the endoplasmic reticulum and covered by the adipocyte-specific perilipins [23]. The lipid droplet should not be perceived as a static storage site for fat; rather, a recent proteomic analysis of the lipid droplet suggests that it forms an important platform for signaling events [24]. Moreover, even in a chronically overfed state, the lipid droplet is in a permanent flux between lipogenesis and lipolysis [25]. The change in lipid droplet size induces a fluctuation in cell size throughout life that can be extreme, ranging from roughly 20 μm in diameter in the depleted state to 200 μm in a fully lipid-laden adipocyte. A final hallmark of a fully differentiated adipocyte is the presence of an insulin-responsive vesicular storage system harboring the GLUT4 glucose-transporter proteins [26]. Stimulation of the adipocyte with insulin leads to a rapid redistribution of the GLUT4 transporters, and concomitant initiation of fusion between these storage vesicles and the plasma membrane, thereby conferring insulin responsiveness on the process of glucose uptake by these cells [27].

Adipogenesis: a tale of three kings

At the transcription level, these profound morphological changes associated with the maturation of the preadipocyte are a finely controlled affair involving the interplay of several families of transcription factors (Figure 1). These include PPAR-γ, CCAAT/enhancer-binding

proteins (C/EBP)-α, -β and -δ, and sterol regulatory element-binding protein 1/adipocyte determination and differentiation factor 1 (SREBP-1/ADD1) [28]. The nuclear hormone receptor PPAR-γ is a transcription factor functioning as an obligate heterodimer with the retinoid X receptor [29]. Expression of PPAR-γ in fibroblasts is sufficient to induce adipogenic differentiation [30], and adipose-specific or hypomorphic PPAR-γ-knockout mice are severely lipodystrophic [31,32]. Therefore, PPAR-γ is widely considered to be the master adipogenic transcription factor. Indeed, ligands of PPAR-γ, such as the antidiabeteic thiazolidinediones (TZDs) troglitazone and rosiglitazone, and the naturally occurring agonist 15-deoxy-$\Delta^{12,14}$-prostaglandin J2, all induce adipogenesis in cells expressing PPAR-γ [33–36]. Interestingly, the metabolic cofactor of PPAR-γ, PPAR-γ coactivator (PGC)-1α is itself a transcriptional target of p53 [37]. Conversely, the aforementioned PPAR-γ agonists have also been shown to induce p53-dependent apoptosis in tumor cells, thereby providing an early example of the interconnection between metabolic and p53-signaling pathways [38–41].

Meanwhile, the C/EBP-family, albeit not unique for the adipocyte, also appears to play an important role in adipogenesis. C/EBP-β (possibly in synergy with C/EBP-δ) induces the transcription of PPAR-γ in the preadipocyte [42,43], thereby triggering full-blown adipocyte differentiation. In the context of this review, it is worthwhile to note that p300/CREB-binding protein-associated factor/general control nonderepressible 5 (PCAF/GCN5) is a direct target of p53 activity [44]. PCAF-dependent acetylation of C/EBP-β is in turn involved in unlocking the adipogenic potential of this transcription factor [45]. Conversely, PCAF is itself also a regulator of p53-transcriptional activity, locking the two in a positive-feedback loop [44,46]. Another C/EBP family member, C/EBP-α, synergizes with PPAR-γ in a positive feedback loop in the regulation of the later stages of adipogenesis, such as the induction of the GLUT4 glucose transporter and adiponectin, and the maintenance of the adipocyte phenotype through a sustained elevation in levels of PPAR-γ [47,48]. With the onset of C/EBP-α, the adipocyte matures visibly, as can be seen by the formation of lipid droplets in the perinuclear region [49]. Interestingly, expression of C/EBP-α is also regulated by a transcriptional target of p53 activity, the transcription factor hematopoietic zinc-finger (Hzf) [50,51]. Combined, the regulation of both these adipogenic C/EBP transcription factors by p53 target genes provides an interesting mechanism for the regulation of the metabolic settings of the organism by the p53 master transcription factor.

A final important adipogenic transcription factor to be considered here is SREBP1: a transcription factor predominantly involved in regulating cholesterol homeostasis [52]. When cholesterol is abundant, this transcription factor is anchored to the endoplasmic reticulum through the ER retention protein Insig (insulin-induced gene) [53] and the escort-protein SREBP cleavage-activating protein (SCAP) [54]. A drop in cholesterol levels allows the release of the SREBP1/SCAP-complex from Insig followed by translocation of this complex to the Golgi complex. Within the Golgi, proteolytic processing events release the basic helix–loop–helix dimerization and DNA-binding domain of SREBP1. This soluble, transcriptionally active cleavage product, nSREBP1, translocates to the nucleus where it initiates transcription of SREBP1-responsive genes, such as *PPAR*-γ, leading to lipogenesis [55] and also to the production of an endogenous ligand for PPAR-γ activation [56]. In this way, SREBP1-activation potently enhances PPAR-γ activity, locking the two in a positive feed-forward loop. An alternative, hormonal pathway mediating SREBP1-transcriptional activation involves PKB-mediated, rapamycin-sensitive, mammalian target of rapamycin complex (mTORC) activation, which is responsible for the insulin-induced increases in lipogenesis [57]. In concert, these transcription factors induce the collection of genes leading to maturation of the adipocyte-precursor to its fully differentiated lipid-laden form.

Living in the fat lane

As much as this specialized system has evolved to deal with free fatty acids and store fat, it seems reasonable to assume that a price must be paid for the protection offered. More than any other cell type in the body, cells in the fat depot are confronted with extreme levels of free fatty acids, cholesterol and the effects of lipotoxicity [58]. To give an inkling of the challenges faced in the fat depot, in blood, the levels of free fatty acids range in the area from 0.2 to 0.4 mM and even a small increase in these levels is associated with a reduction in insulin release by the β-cell and a marked loss of insulin sensitivity in muscle [59–61]. In comparison to blood it is difficult, if not technically impossible, to obtain a reliable measurement of free fatty acids in the interstitial space between adipocytes within the fat depot. However, that it will be higher than 0.4 mM, even staggeringly high, seems quite a safe bet. The deleterious effects of prolonged exposure to these conditions in the fat depot are readily apparent as committed preadipocytes from older animals demonstrate changes in their ability to undergo adipogenic differentiation and lipogenesis [62–64].

Remarkably, even under such adverse conditions, adipocytes and preadipocytes turn out to be fairly long-lived cells. Recently, Arner and coworkers made clever use of ^{14}C derived from nuclear bomb tests. Levels of this radionucleotide in the atmosphere markedly increased during the Cold War and have dropped exponentially since the Test-Ban Treaty in 1963. As DNA is synthesized at the moment of cell division and is stable afterwards, comparing the levels of ^{14}C incorporated into the DNA during its synthesis to known atmospheric ^{14}C levels can mark the date a cell was born. Their data suggest that the number of adipocytes in an adult is set during childhood and adolescence [65]. Furthermore, they found that roughly half of all adipocytes are replaced every 8.3 years irrespective of the level of obesity (and associated increases in levels of free fatty acids). Interestingly, this implies a theoretical possibility for some adipocytes (~0.5%) to not turn over at all in the span of a human life.

In an adult, it must now be concluded that it is mainly hypertrophy of differentiated, committed preadipocytes as opposed to hyperplasia from stem cells and subsequent *de novo* adipogenesis that is responsible for the expansion of the fat depot under high caloric intake in an adult [65]. In essence, after the initial waves of cellular hyperplasia from pluripotent and multipotent cells, and commitment to the adipocyte lineage during youth and early adolescence, a large proportion of the cells lie dormant in a semi-senescent state in the fat depot until cued for enlargement (Figure 1). Kirkland *et al.* suggest that these preadipocytes account for 15–50% of the total cell population in the fat depot [66,67]. Importantly, it suggests that these preadipocytes must maintain viability and the ability to differentiate into a lipid-laden adipocyte from early adolescence onwards and throughout life. As a consequence, a certain amount of innate tolerance to the cellular and oxidative damage caused by high levels of free fatty acids can be expected as a trait of all cells that have residence in the fat depot.

Studies performed by our collaborators and at our laboratories uncovered a further important principle when we compared obese individuals that succumb to insulin-resistance, and potentially diabetes, with obese individuals that maintain insulin sensitivity. We found that the insulin-resistant group are characterized by the larger presence of ‘small’ differentiated adipocytes, suggesting the final stages of the adipogenic conversion process are hampered [68–70]. As shown in the light blue graph inset of Figure 1, these ‘small’ adipocytes form a major component of any fat depot and can perhaps best be seen as immature adipocytes with little lipid and very small droplets. These cells do float when suspended, thereby clearly distinguishing them from other cells in the fat depot, such as fibroblasts, committed preadipocytes and macrophages. It seems intuitive and plausible that these cells form a transitional stage between the more fibroblastic preadipocyte and a fully engorged, lipid-laden adipocyte. In that light, our hypothesis is that these cells fail to make the final adipogenic step

in insulin-resistant individuals. By failing to engorge themselves on lipids, the problem persists, prompting more cells to undergo terminal differentiation, and at the same time exposing the organism to an excessive lipid load with many deleterious side-effects to bear. However, it could be argued that in theory these small cells could also originate from a large adipocyte cell after undergoing massive lipolysis, or they could form a completely separate end point, independent from the large adipocytes and without any movement between these cellular populations whatsoever. Future research will be needed to tease apart these different possibilities.

With that caveat in mind let us for now resume our train of thought following the assumption made in Figure 1. The beneficial protective effects these cells have in permitting a happy, healthy 'Burgundian Life' [71] to the obese individual should then be readily apparent. For these individuals have a reservoir of cells at their disposal that maintain viability and the ability to differentiate to a fully enlarged lipid-engorged state. As a consequence, they can mobilize these cells to deal with the additional lipid and cholesterol load to protect these individuals from the lipotoxic and inflammatory effects of free fatty acids, ectopic lipid accumulation and the ER-stress caused by cholesterol. Failure to do so results in the obese individual succumbing to diabetes and its associated morbidities. It is of interest to note in this discourse the observation made by Blüher *et al.,* of an even more profound heterogeneity between small and large adipose cells in the adipose-specific insulin receptor-knockout mice, although the molecular mechanisms remain enigmatic [72]. In continued close collaboration with Reaven, Smith and coworkers at the Universities of Stanford, USA, and Gothenburg, Sweden, we took this material from our previous studies [68–70,73] and cross-referenced it against all other publicly available arrays using gene-set enrichment analyses. This approach gives a very direct, unbiased readout of which signaling pathways are involved in a biological phenomenon as the data from the studies are matched against all possible array-information in the public domain, without being influenced by the scientist. As an example, in this case, by comparing expression profiles between groups of equally obese people separated into groups of insulin-resistant and insulin-sensitive subjects, one can assume that differences in gene expression between these individuals would match up with signaling pathways characterized in other array studies, such as a study into immortalization of cancer cells, thereby shedding some light, even if only correlative, at the molecular mechanisms involved in turning the small adipocytes into fully lipid-laden cells. Much to our surprise, alongside the expected matches with studies on adipogenesis or studies on the insulin-sensitizing effects of TZDs, our analyses also matched profiles of studies describing several well-known canonical p53-transcriptional targets, such as *p21*^{waf1} [74–76], as well as lesser-known targets, such as oxysterol-binding protein (OSBP) [44], suggesting an involvement of the p53 cell cycle-control pathway in the regulation of this adipogenic conversion process. Unfortunately, data in the literature are sparse, and only a few mouse models have been studied in any detail with respect to this question. For one, it has been demonstrated that p21^{waf1} is crucial for protecting the hypertrophied adipocytes in diet-induced obese mice from apoptosis [77]. Another important study by the same group from the University of Tsukuba, Japan, identified p53 as a component in a negative-feedback loop preventing excessive fat accumulation in the adipocytes of obese, leptin-deficient mice [78]. Unfortunately, however, no measurement was made on the size distribution of the adipocytes in this latter study. It is to be hoped that these studies will be revisited in the near future and complemented with an analysis of cell-size distribution, which will provide valuable additional information. Perhaps the most intriguing study in this respect involves Donehower's hypermorphic p53 mice: these mice exhibit altered fat depots and dyslipidemia reminiscent of accelerated aging [79,80]. However, it remains to be determined to what extent this mouse model is a true hypermorph of p53 activity and not a p53-mutant inducing transcription of some, but not all transcriptional targets of p53 [81]. Another caveat is that all these studies are performed on whole-body mouse knockout models, thus making it very difficult to attribute alterations in the metabolic settings of these mice directly to the fat depots. Irrespective of these

considerations, these studies do seem to corroborate a role for p53 in the regulation of adipogenesis throughout adult life and, thereby, its profound influence on the metabolic setting of the organism.

The p53 transcription factor is predominantly known as a critical regulator of cell cycle arrest and apoptosis in cells when confronted with a genotoxic insult such as UV-damage or ionizing radiation [82,83]. Indeed, it has been estimated that aberrant p53 signaling is involved in at least 50% of human cancers [84,85]. It is this role of p53 in preventing neoplasias that gave rise to its nickname ‘guardian of the genome’ [86]. Notwithstanding the importance of the p53 tumor suppressor in safeguarding the organism against cancer, what could possibly be its role in the protection against age- or obesity-induced Type 2 diabetes?

A recent major shift in paradigm in the field of p53-signaling provides an important clue to the resolution of this theoretical conundrum. Currently, it is suggested that aside from its key apoptotic role in the face of acute and extreme genotoxic insults, low levels of p53 expression, induced by the rigors and strains of daily life, make an important contribution to the maintenance of the organism [81,87]. Far from promptly inducing apoptosis, a low but continuous level of p53 activity actually induces a cytoprotective set of genes involved in oxygen radical scavenging and cellular maintenance [88,89]. Both free fatty acid-derived ROS and cholesterol-derived ER stress are exactly the ‘rigors and strains of daily life’ that activate p53 in this manner. In the remainder of this review, we will introduce some of the basic tenets of p53 signaling and discuss the potential for several of its transcriptional targets to either protect the preadipocytes from oxidative stress or to provide the immature, small adipocyte with an enhanced lipogenic capacity, thereby greatly improving the ability of the cell to act as a lipid sink. Please see Figure 2 for a depiction of the following hypothesis.

The guardian of corpulence

Control over the p53-signal transduction pathway is achieved by two major p53-binding and regulating proteins: Mdm2 and Mdm4 [90]. *Mdm2* was identified in the early 1990s as a gene present on the double-minute amplicon of a spontaneously transformed mouse cell-line [91]. Several studies identified Mdm2 as a major regulator of p53 through its ability to bind p53 and shuttle it out of the nucleus, thereby preventing its ability to activate transcription [92–94]. Furthermore, Mdm2 also acts as an E3-ubiquitin ligase and thereby destabilizes the p53 protein through ubiquitin-mediated proteasomal degradation [95,96]. However, a recent paper describing a knock-in mouse expressing mutant Mdm2 (which cannot degrade p53) demonstrates that simple binding is not sufficient for inhibition of p53-activity [97]. This manuscript highlights the prime importance of this ubiquitin-ligase function of Mdm2. As the *Mdm2* gene itself is a transcriptional target of p53, the two are locked in a negative-feedback loop ensuring p53 levels are maintained at very low levels (Figure 3). The vital importance of this activity is demonstrated in the *mdm2* homozygous null mice, which die *in utero* owing to p53-induced apoptosis [98,99].

A homologous protein, Mdm4 (originally termed MdmX), was identified in the mid-1990s in a screen for p53-binding proteins [100,101]. In contrast to Mdm2, Mdm4 does not cycle in and out of the nucleus on its own and does not act as a ubiquitin ligase [102]. Surprisingly, however, Mdm4-knockout mice are embryonically lethal owing to massive cell cycle arrest, and akin to the Mdm2-knockout mice, this lethality can also be prevented in a *p53*-homozygous null background [103]. Combined, these studies identified the Mdm2 and Mdm4 proteins as crucial non-redundant regulators of p53 [104]. Upon disruption of the complex between p53 and the Mdm proteins, levels of nuclear p53 rapidly increase and p53-mediated transcription is initiated.

Transcriptional activity of p53 is further regulated through deacetylation by Sirt1. These NAD^+-dependent protein deacetylases appear to mimic the cell-survival and life-prolonging effects of caloric restriction by attenuating p53-activation, promoting fat mobilization and preventing adipogenesis [105–107]. Vital to the effects of p53 is its activity as a transcription factor initiating the synthesis of a wide range of proteins. One of the best known targets of p53-transcriptional activity is $p21^{waf1}$ (wild-type-p53-activated factor), which acts as a cell cycle inhibitor and protects cells from undergoing apoptosis [74–76].

Another important point of cross-talk between p53 and metabolic signaling pathways is AMPK. Several reviews by Hardie and coworkers present a detailed description of this major metabolic regulator of cellular energy homeostasis [108–110]. In the context of this review, it is important to note that AMPK can stabilize and activate p53 by phosphorylation of Ser15 [111,112]. Through this mechanism, a checkpoint is emplaced ensuring arrest of the cell cycle in periods of glucose deprivation. Indeed, as such, AMPK-mediated activation of p53 may be a far more common event in the life of a cell than ionizing radiation-mediated p53-stabilization and apoptosis. Remarkably, data by Karin *et al.* illustrate that the reverse holds true as well: active p53 induces the transcription of sestrins [113], which subsequently mediate the autophosphorylation and activation of AMPK and concomitant inactivation of mTORC, the rapamycin-sensitive metabolic regulator of protein synthesis. In essence, this pathway is thought to ensure arrest of protein synthesis when further cellular division is halted by p53. Furthermore, PRKAG2, the regulatory subunit of AMPK, is itself a transcriptional target of p53 activity [44], suggesting the two pathways are intimately interconnected in positive-feedback mechanisms (Figure 3).

All about stress

The previously mentioned sestrins are members of an ancient, evolutionarily conserved family of proteins involved in the regeneration of the thiol-containing peroxidase-scavenging proteins. As such, their original role appears to be in forming an important line of defense against ROS [114]. In addition, this cytoprotective effect against ROS may well be of significant functional importance in protecting the preadipocyte against the oxidative damage associated with lipotoxicity. Another very interesting p53 target gene with regard to protection against oxidative stress is Tp53-induced glycolysis and apoptosis regulator (*TIGAR*) [115]. TIGAR shows significant homology to the bisphosphatase domain of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (Fru-2,6-P_2) and indeed, overexpression of *TIGAR* causes a decline in intracellular levels of Fru-2,6-P_2. Fru-2,6-P_2 is both a positive allosteric effector of 6-phosphofructo-1-kinase and also an inhibitor of fructose-1,6-bisphosphatase; thereby the net effect of TIGAR-mediated reductions in levels of Fru-2,6-P_2 is a reduction of glycolysis and redirection of the accumulated glucose-6-P into the pentose phosphate shunt. The generation of NADPH and increases in reduced glutathione as a resultant of the pentose phosphate shunt also serve to protect the cell from oxidative damage [87,115]. Furthermore, the contribution of p53-induced target genes, such as glutathione peroxidase (*GPXI*) [116] and aldehyde dehydrogenase (*ALDH4A1*) [117], in protecting the cell against oxidative damage [89] is well established. In essence, this combined transcriptional profile of p53 could serve the cells residing in the fat depot well in conferring protection to one of the major mediators of lipotoxicity: ROS.

The StARD of the story: a thought on oxysterol

Aside from providing cytoprotection from ROS, some recent observations suggest p53 could, in theory, provide the adipocytes with a more direct handle on free fatty acids and cholesterol by enhancing the lipogenic capacity of these cells. Thereby, lipotoxicity-induced p53 activation would aid these cells in an attempt to attenuate the sources of stress in the fat depot directly

(see Figure 4 for a depiction of this thought). Wei and coworkers identified two p53-target genes, *StARD4* and *OSBP*, in a genome-wide map [44]. StARD4 was originally identified as a mediator of intracellular lipid metabolism under the control of SREBP1 transcription factors [118,119] and belongs to a family of steroidogenic acute regulatory proteins that are involved in transporting the water-insoluble cholesterol across the cytosol to the mitochondrion where it can be converted into the potent signaling molecule oxysterol [120–122]. How oxysterol interacts with metabolic settings in the body is currently not understood in detail. However, this more soluble derivative of cholesterol acts in part through its cognate receptor liver X receptor (LXR) that, in turn, has been demonstrated to induce PPAR-γ upon its activation [123–125].

In light of the observations made on our human subjects [61–63], the involvement of this pathway starts making some sense: for, even though the LXR receptors are not required for the initiation of adipogenesis *per se*, these receptors are involved in initiating age- and diet-induced adipocyte hypertrophy. LXR-knockout mice demonstrate a reduced adipocyte size and do not gain weight when fed a high-fat diet [125,126]. Furthermore, and importantly, oxysterol levels in the serum of subjects markedly associates with obesity, oxidative stress and components of the metabolic syndrome in adolescents [127]. Furthermore, it is of interest to note that it has recently been shown that a potent proadipogenic enzyme, 11β-hydroxysteroid dehydrogenase (11-HSD), catalyzes the reduction of 7-ketocholesterol leading to the accumulation of the oxysterol 7β-hydroxycholesterol in the adipocyte [128–131]. Furthermore, oxysterol allosterically activates an ER-resident enzyme, acyl-coenzyme a:cholesterol acyl-transferase (ACAT) [132,133]. This enzyme converts cholesterol into cholesterol esters, which are stored in the neutral core of the lipid droplet [134,135]. Combined with the initiation of LXR-mediated lipogenesis and enhanced lipid droplet biogenesis this creates a cholesterol-sink, thereby liberating the SREBP-transcription complex from the negative feedback imposed by cholesterol and a further enhancement of the lipogenic capacity of the cell. These indirect boosts of LXR- and PPAR-γ-activity downstream of p53 may enhance the adipogenic conversion of preadipocyte or prompt a small adipocyte to engorge itself with lipids (Figure 4). Based on the observations mentioned previously, oxysterol certainly merits further study as an attractive theoretical mediator of these effects.

Another p53-target with potential close association to this oxysterol-signaling pathway is OSBP. OSBP was originally identified as a putative oxysterol receptor involved in maintaining cholesterol and lipid homeostasis [136]. However, these roles are actually mediated through the previously mentioned LXR and SREBP1 [137]. OSBP is the founding member of a large family of 16 predicted proteins [138,139]. These family members have several different membrane– and protein–protein interaction domains in conjunction with their OBD, but their function remains enigmatic. It has been demonstrated that OSBP potentiates SREBP1 signaling and is crucial for the insulin-induced induction of SREBP1 and lipogenesis [137]. Furthermore, it has also been demonstrated that LXR-transcriptional activity is unaffected by OSBP [137]. Thereby, the combined effect of OSBP on both SREBP1 and LXR-PPAR-γ enhances these lipogenic transcription factors even further. It seems these novel p53 targets are capable of orchestrating a finely tuned sequence of events within the adipogenic process, initiating the genesis of the lipid droplet and strongly potentiating lipogenesis. Thereby, in contrast to the destruction inherent to an apoptotic process, these cells could rely on the p53 transcription factor to build themselves into a lipid-sink with a large capacity to deal with free fatty acids in the protection of the organism.

Future perspective

Current research in our laboratories and studies by our colleagues are aimed at verifying and validating key components of this hypothesis. In particular, we aim to elucidate the effects of

alterations in p53 signaling on animal metabolism in collaboration with Dr Lozano (Houston, TX, USA), by employing available mouse models such as the floxed *mdm2* and *mdm4* mice. Naturally, we are also very interested in studying the physiological role of oxysterol in the maturation of adipocytes and the regulation of adipocyte cell size, and would welcome anyone interested in collaborating with us on this topic. The suggested fundamental link between p53, cytoprotection and adipogenesis provides an interesting new way of thinking about signaling pathways involved in age-related afflictions such as diabetes and cancer. In the coming years, if proven correct, a deeper understanding of these pathways can provide attractive new avenues for pharmacological intervention influencing adipocyte mobilization during times of increased requirement for lipid storage, the prevention of the ectopic deposition of fat with advancing age, and the onset of Type 2 diabetes.

Executive summary

- Adipocytes are of vital importance in protecting the organism against the deleterious effects of ectopic lipid deposition.
- Preadipocytes must survive the lipotoxic conditions in the fat depot throughout adult life and maintain the ability to differentiate.
- Data obtained by our studies suggest an involvement of the p53-transcription factor in protecting obese individuals from diabetes.
- The antioxidant function of the p53-transcription factor may confer protection from the oxidative damage associated with lipotoxicity.
- Furthermore, transcriptional targets of p53 could induce lipogenesis and enhance the lipid-storage capacity of adipocytes.
- Combined, p53 may aid the cells residing in the fat pad in dealing with the effects of lipid and cholesterol overload, thereby protecting the organism from ectopic lipid deposition and lipotoxicity.

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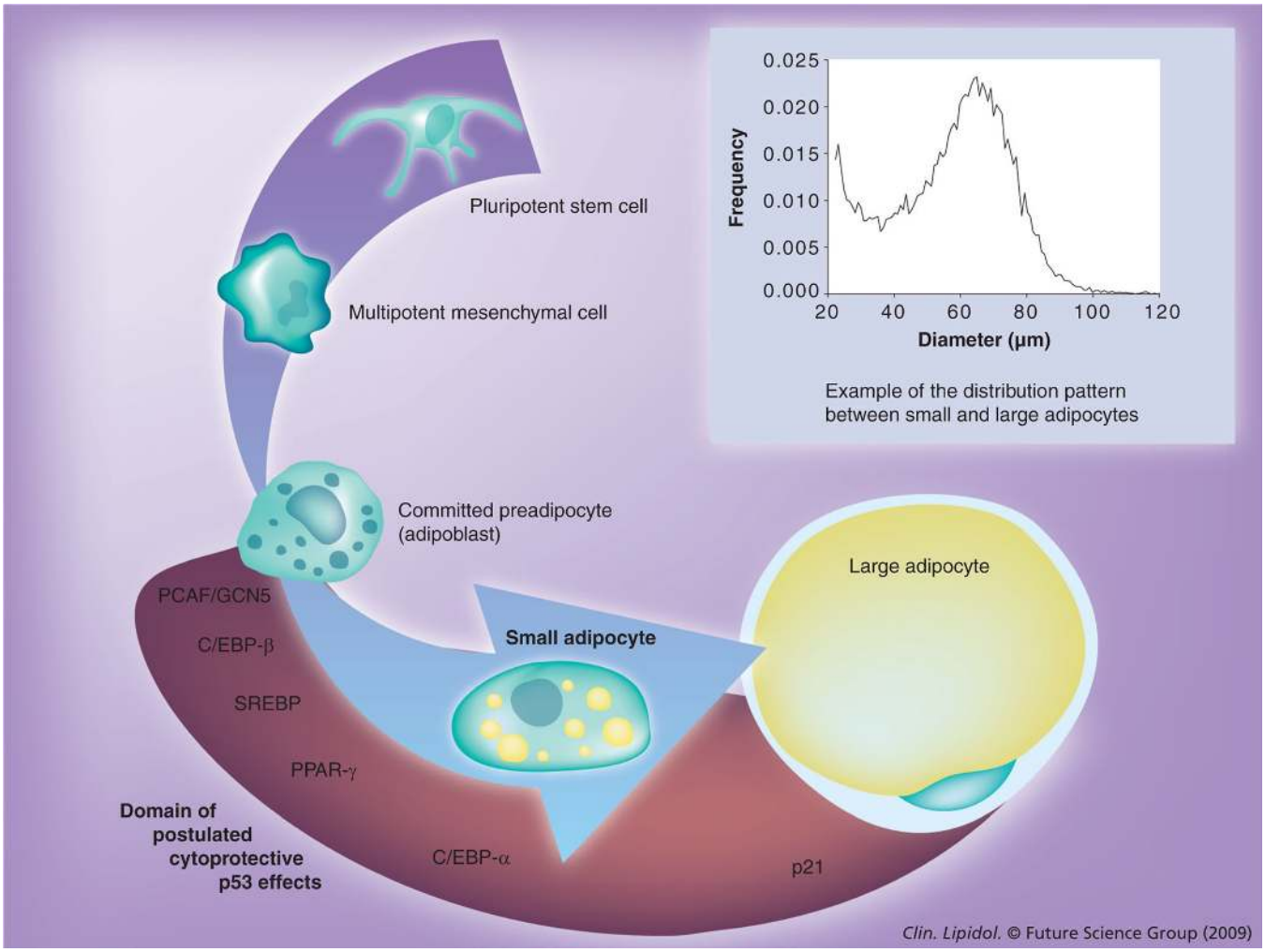

Figure 1. Stages in the adipogenic conversion process

The insert depicts a typical example of the distribution between the small and large adipocytes we observe in an organism. Initial pluripotent stem cells are committed to the mesenchymal lineage. Subsequent differentiation steps lead to loss of potency and the ability to divide in the formation of a fully committed preadipocyte cell. Upon initiation of adipogenesis these cells accumulate small lipid droplets in the perinuclear region and undergo marked alterations in cellular morphology and transcriptional activity. Ultimately, these small lipid droplets engorge themselves with lipids and become enlarged, fully lipid-laden adipocytes. The cytoplasm and nucleus of these rounded cells are squeezed by the enormous lipid droplet into a narrow area just underneath the plasma membrane. The relative position of several key transcription factors involved in adipogenic conversion (PCAF/GCN5, C/EBP-β, SREBP, PPAR-γ and C/EBP-α) is depicted at the relative position of their action during adipogenesis. The red shaded area and arrow indicate the time wherein we postulate p53 to be active in the manner described in the text. It is of interest to note that in the initiation phase of adipogenesis in the 3T3-L1 cell line, after the contact-inhibited cell-cycle arrest and clonal expansion phase, a large amount of cells are lost owing to apoptosis. Indicative of an involvement of p53-signaling pathways as this may be, it is at present unclear if these stages in 3T3-L1 adipogenesis (a semi-transformed cell-line) accurately reflect the process of adipogenesis *in vivo*. C/EBP: CCAAT/enhancer-binding protein; GCN5: General control nonderepressible; PCAF: p300/CREB-binding protein associated factor; SREBP: Sterol regulatory element-binding protein.

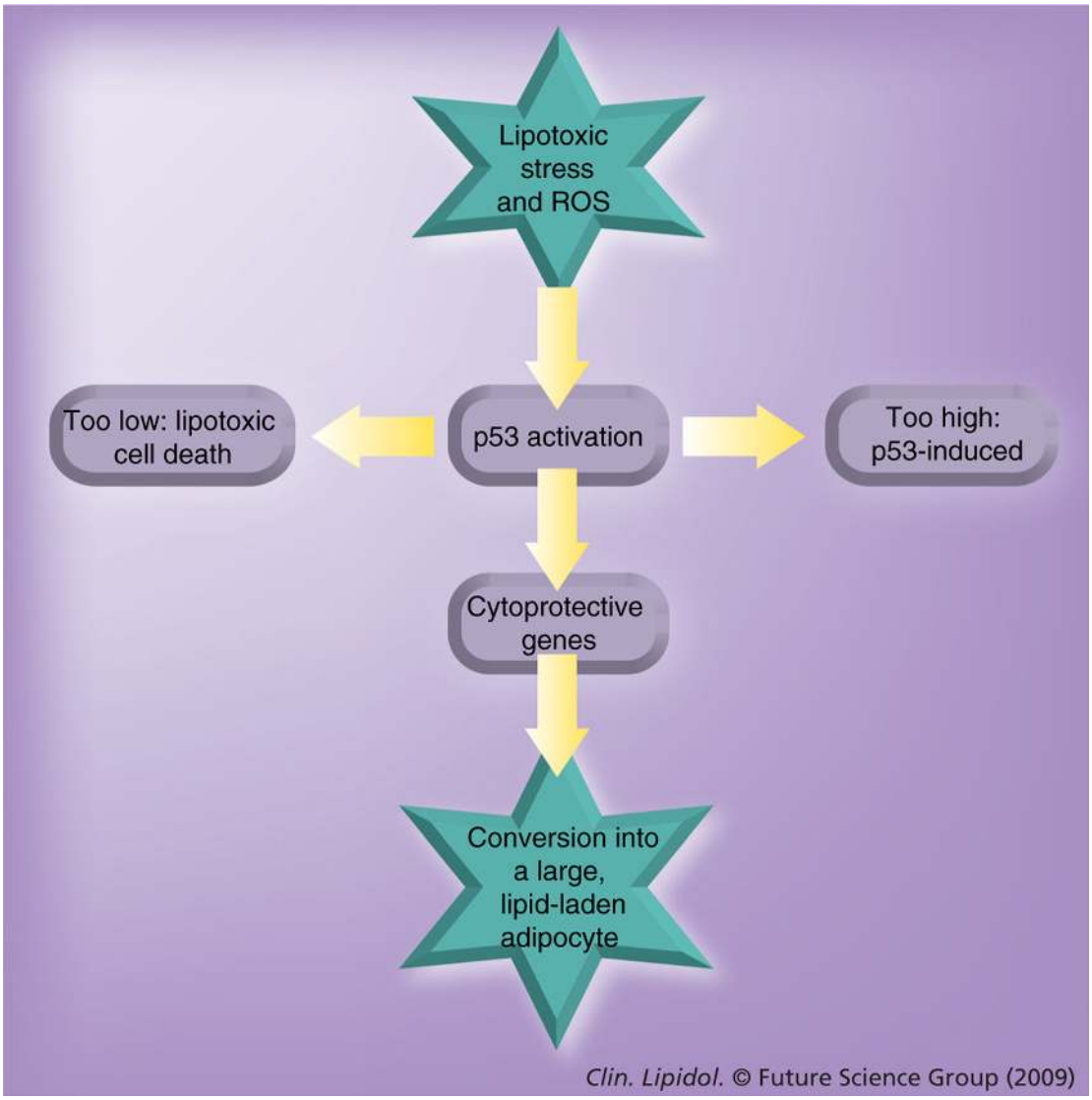

Figure 2. The underlying hypothesis on the role of p53 in adipogenesis

Exposure to free fatty acids in the interstitial fluid will activate p53 through lipotoxic stress and ROS-induced cellular damage. Is the level of p53-activation too high and persistent? The tumor-suppressor p53 will eventually induce apoptosis, leading to loss of the cell. Is the level of p53 activation too low? There will not have been an effect of this 'guardian' at all and the cell will ultimately succumb to the damage induced by reactive oxygen radicals and lipotoxic effects. Only when the level of p53 activation is 'just right', will it confer protection to the small adipocytes by the induction of reactive oxygen-scavenging genes and possibly even by enhancing the lipogenic capacity of these cells. Thereby, p53 activation, when properly controlled, will ensure the clearance of lipotoxic stress, protecting not just the cell, but once again the organism as a whole. However, it is worthy to note for consideration, that in our present-day society 'just right' may have shifted considerably from our original evolutionary settings.

ROS: Reactive oxygen species.

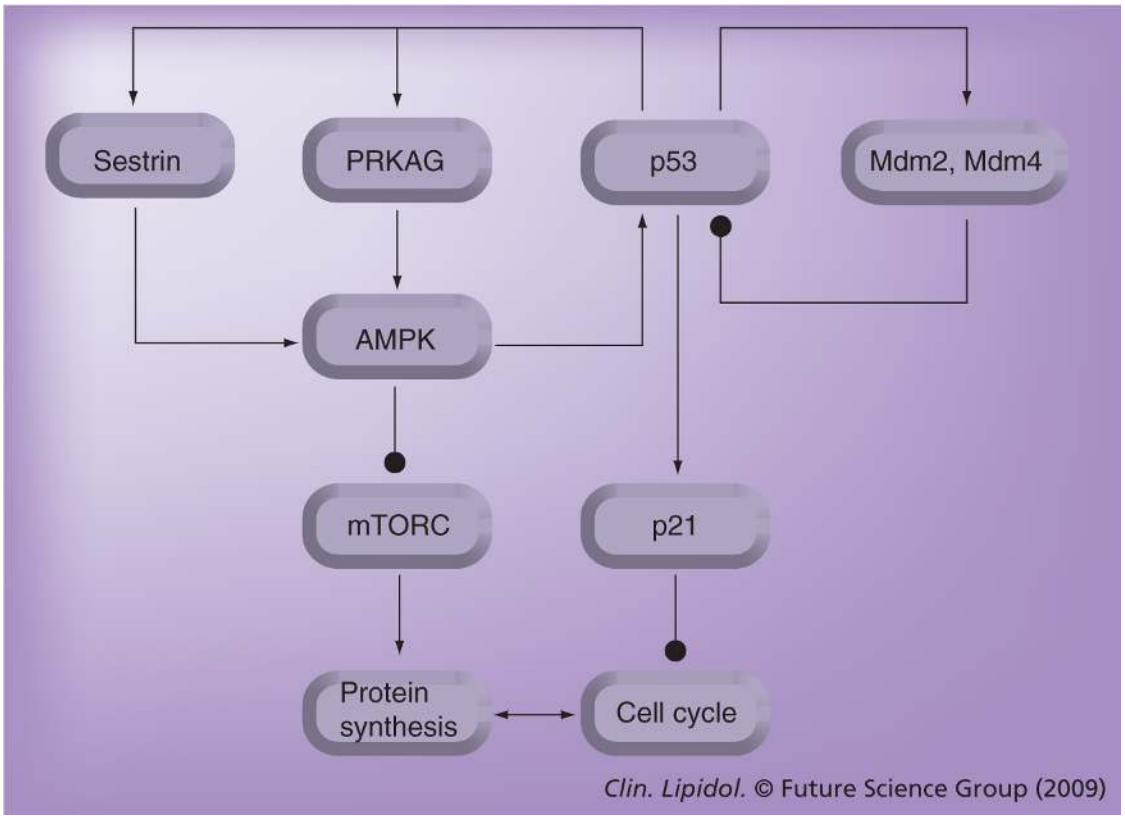

Figure 3. Negative- and positive-feedback loops involved in p53 signaling

Negative- (closed arrow –•) and positive- (open arrow →) feedback loops involved in p53 signaling are highlighted and the manner in which they intertwine to synchronize regulation of the cell cycle with the regulation of protein synthesis. Indicated are classical components of the p53-mediated cell-cycle arrest pathway: Mdm2, Mdm4 and the p53-effector $p21^{waf1}$; essential components of the metabolic fuel-sensing pathway: AMPK and mTORC; and the recently positioned sestrins, as discussed in the text.

mTORC: Mammalian target of rapamycin complex.

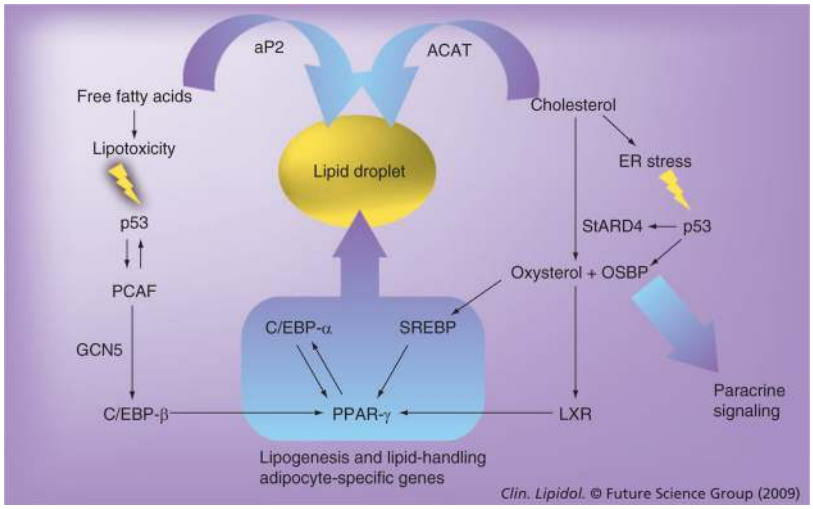

Figure 4. Potential involvement of p53 activity in adipogenesis, as hypothesized in the text

Free fatty acid-mediated lipotoxity and associated oxidative stress and cholesterol-overload with associated ER stress can both induce p53 activation. Several recently identified transcriptional targets of p53, such as PCAF, StARD4 and OSBP, impinge on known components of the transcriptional machinery governing adipogenic conversion. In particular, StARD4 is of interest as it contributes to the synthesis of oxysterol. This powerful signaling molecule can mediate paracrine signaling and acts on a known, but enigmatic component of adipogenesis, the LXR receptors. The net increase in lipogenic activity and the genesis of the lipid droplet in these cells will subsequently provide a sink for the excessive amounts of free fatty acids and cholesterol (represented by curved arrows) governed by the aP2 fatty acid transporter and the ACAT mediator of cholesterol esterification. This ultimately leads to removal of the stresses that initiated p53-activation and termination of the p53-signal (prior to the initiation of apoptotic processes). It is noteworthy that the respective position of StARD4, OSBP and PCAF is only due to diagrammatic constraints: either lipotoxicity or ER-stress can induce these transcriptional targets of p53 through the activation of p53-mediated transcription. A detailed description of this model and its respective components can be found in the text along with accompanying references. ACAT: Acyl-coenzyme A:cholesterol acyl-transferase; C/EBP: CCAAT/enhancer-binding protein; ER: Endoplasmic reticulum; LXR: Liver X receptor; OSBP: Oxysterol-binding protein; PCAF: p300/CREB-binding protein associated factor; SREBP: Sterol regulatory element-binding protein.