COMMENTARY

Dropping in on the lipid droplet- tumor protein D52 (TPD52) as a new regulator and resident protein

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ABSTRACT

Lipid droplets are essential for both the storage and retrieval of excess cellular nutrients, and their biology is regulated by a diverse range of cellular proteins, some of which function at the lipid droplet. Numerous studies have characterized lipid droplet proteomes in different organisms and cell types, and RNAi whole genome screening studies have examined the genetic regulation of lipid storage in \textit{C. elegans} and \textit{D. melanogaster}. While tumor protein D52 (TPD52) did not emerge from earlier studies as a strong candidate, exogenous expression of human TPD52 in cultured cells resulted in significantly increased numbers of lipid droplets, and oleic acid supplementation increased TPD52 detection at both lipid droplets and the Golgi apparatus. These results suggest that direct testing of proteins that are infrequently but recurrently identified in proteomic and RNAi screening studies may identify novel lipid droplet regulators. While the analysis of these possibly lower-abundance or itinerant lipid droplet proteins may be more technically challenging, such proteins could facilitate a more detailed interrogation of emerging aspects of lipid droplet biology.

KEYWORDS

Golgi apparatus; lipid droplet; lipogenesis; perilipin; tumor protein D52

The lipid droplet (LD) has progressed from being considered an inert cellular organelle functioning as a storage depot to one that is understood to be highly responsive to the metabolic requirements of the cell.\textsuperscript{1} Indeed, the long evolutionary history of LDs suggests that they represent a basic prerequisite for organism survival.\textsuperscript{1} In humans, most excess fatty acids are stored and mobilised from large unilocular LDs within adipocytes, which can be up to 100 $\mu$m in diameter.\textsuperscript{2} Nonetheless, the storage capacity of adipose tissue is frequently exceeded in conditions such as obesity, leading to aberrant lipid accumulation in smaller LDs within skeletal muscle and liver cells.\textsuperscript{2} As dysfunctional lipid storage plays a key role in conditions as diverse as diabetes, inflammation, obesity and cancer, adipocytes and LDs are being increasingly investigated as drug targets.\textsuperscript{3}

The LD is unique among cellular organelles in that it is bound by a phospholipid monolayer.\textsuperscript{1} This amphipathic phospholipid coating serves to emulsify the hydrophobic core of triglycerides and cholesterol esters within an aqueous cytosol.\textsuperscript{4} Many proteins have been reported to be associated with LDs,\textsuperscript{5,6} where the amphipathic nature of the membrane (hydrophilic facing the cytosol, hydrophobic at the core) is predicted to represent an important determinant of how proteins associate with the LD.\textsuperscript{5} Some LD proteins may be regular residents, while others may drop in and out, and also function within other cell compartments.\textsuperscript{1,5} While it is perhaps tempting to conclude that the major regulatory proteins have been identified, Kamili et al. recently reported a new protein regulating LD biology.\textsuperscript{7} This protein is tumor protein D52 (TPD52), a protein first discovered through its overexpression in cancer some 20 years ago.\textsuperscript{8}

Historical background

Since its identification, the functions of tumor protein D52 (TPD52) have been largely a mystery. TPD52 represents one of 4 paralogues in mammals (Table 1, Fig. 1).\textsuperscript{9} The family includes 3 genes (\textit{TPD52}, \textit{TPD52L1}, and \textit{TPD52L2}) with broad but incompletely overlapping tissue expression, and one gene (\textit{TPD52L3}) whose expression is largely restricted to the testis (Table 1).\textsuperscript{9} The functions of TPD52-like proteins have been difficult to predict, as their sequences have been poorly predictive of function. TPD52 overexpression in different cancer types...
has been broadly reported, as have regulatory functions within signaling pathways.9,10 However, these types of results can be demonstrated for many cellular proteins, so apart from the analysis of TPD52 functions in secretory processes,11,12 progress in understanding TPD52’s molecular functions has been slow.10 A lack of structural information has also made it difficult to predict how TPD52 contributes to macromolecular complexes, and what additional functions it might have.

**Early evidence**

While Kamili et al.7 were the first to demonstrate a LD function for TPD52, these results were not entirely unanticipated. However, individual studies linking TPD52 and cellular lipid storage had not drawn previous attention within the literature. The mouse Tpd52 ortholog was reported to be upregulated 5-fold in obese versus lean mouse adipose tissue,13 and knock-down of the ancestral C. elegans TPD52 ortholog F13E6.1 was subsequently associated with reduced lipid storage.14 However, this latter result was not supported by several whole genome RNAi screening studies in D. melanogaster,15-17 although a proteomics study reported a moderate association between the ancestral D. melanogaster TPD52 ortholog CG5174 and LDs.18 In both these C. elegans and D. melanogaster studies,14,18 TPD52 orthologues were identified as anonymous proteins, and the

| Protein  | TPD52 | TPD52L1 | TPD52L2 | TPD52L3 |
|----------|-------|---------|---------|---------|
| Uniprot ID | P55327 | Q16890 | Q43399 | Q96J77 |
| Cytogenetic location of corresponding human gene | 8q21.13 | 6q22.31 | 20q13.33 | 9p24.1 |
| Demonstrated or predicted tissue expression | Broad | Broad | Broad | Restricted |
| Identified in C. elegans/ D. melanogaster | Common ancestral gene/protein identified (F13E6.1, CG5174) |
| RNAi or proteomics screening? | Yes | No | Unknown | Restricted |
| Binds perilipins? | Yes | No | Unknown | Unknown |
| Protein/ ortholog reported within mammalian LD proteome? | Yes | No | Yes | No |
| Major isoforms include 14-3-3 binding site? | No | Yes | No | No |
| Note. *This result may reflect the tissue-restricted expression of TPD52L3.*

![Figure 1](image_url)
nomenclature used suggested no link to mammalian TPD52 proteins (Table 1). Perhaps the closest “near miss” was the identification of TPD52 as a perilipin-1 (PLIN1) binding partner through yeast 2-hybrid screening.19 PLIN1 is a member of the perilipin (PLIN) protein family (previously referred to as the PAT protein family), which are well characterized LD-associated proteins.3 The same yeast 2-hybrid screening that identified TPD52 also identified CGI58,19 a major regulator of PLIN1 function.20 However, when GFP-tagged TPD52 was expressed in 3T3-L1 cells, GFP-TPD52 showed a perinuclear sub-cellular localization, contrasting with the obvious LD co-localization of GFP-tagged CGI58.19 The authors may therefore have considered TPD52 as a screening false-positive, and there was no further published follow up of TPD52 as a PLIN1 partner.

Our group reconsidered these and other results following the publication of Kourtidis et al. in 2010, which described the reliance of ERBB2-positive breast cancer cell lines upon chromosome 17-encoded regulators of lipid metabolism.21 At the time, we were analyzing the effects of TPD52 knock-down in breast cell lines.22 Like Kourtidis et al.,21 we noted that TPD52 knock-down decreased the survival of ERBB2-positive breast cancer cell lines,22 which were characterized by higher levels of LD staining.21 Breast and prostate cancers have been shown to accumulate cytoplasmic LDs,23-25 and ERBB2 expression in breast cancer has been associated with a transcriptional program upregulating fatty acid synthase.26 TPD52 also has a long history of being identified and studied as an androgen-induced gene/protein.27,28 androgen treatment of prostate cancer cells increases cytoplasmic LDs25 and regulates key anabolic enzymes.29 Careful searches of the literature identified previous links between TPD52 and lipid storage,13,14,18,19,30,31 which were bolstered by numerous proteomic studies reporting the TPD52 parologue TPD52L2 as a LD-associated protein (Table 1).32-37 Armed with these collective results, we examined whether TPD52 expression led to increased cellular lipid storage, and whether TPD52 represented be a bona fide partner of PLIN proteins.7

New findings

The approaches taken by Kamili et al.7 were straightforward. Existing cell line models were examined to determine whether TPD52 expression status altered LD numbers, and this was the case in the 2 series of cell lines available (mouse Balb/c 3T3 cells and human MDA-MB-231 cells).7 However, 3T3 cells expressing a related TPD52 protein (TPD52-like 1, or TPD52L1) (Fig. 1) showed LD numbers similar to those in vector controls and parental cells.7 Correspondingly, to our best knowledge, TPD52L1 has not been identified within any reported LD proteome (Table 1). We then studied TPD52-expressing 3T3 cell lines in more detail, and found that these contained more LDs following oleic acid supplementation than did control cells.7 Two of 3 TPD52-expressing 3T3 cell lines also showed significantly increased lipogenesis from both de novo synthesis and exogenous uptake.7 TPD52 partially co-localized with Golgi markers and Plin2/Adrp-coated LDs, with co-localization becoming more pronounced following oleic acid supplementation.7 Interestingly TPD52 showed detectable interactions with both PLIN2/ADRP and PLIN3/TIP47 in the yeast 2-hybrid system (Table 1),7 supporting the earlier identification of TPD52 as a PLIN1 partner.19 However, as TPD52L1 did not detectably interact with PLIN proteins (Table 1), isoform-specific interactions between TPD52 and PLIN proteins may therefore underpin a function for TPD52 in promoting intracellular lipid storage.7

How does TPD52 associate with lipid droplets?

While direct associations with PLINs may mediate TPD52 localization at LDs,7 such associations do not exclude TPD52 also directly associating with the LD. Amphipathic helices may underpin protein associations with LDs and other organelles,4,38 and although TPD52-like proteins include predicted amphipathic helices, their significance is unknown. Other hydrophobic LD targeting motifs identified in lipid-droplet associated proteins39,40 are not predicted in TPD52-like proteins. As TPD52L1 expression in 3T3 cells did not increase LD numbers and TPD52L1 did not detectably bind either PLIN2/ADRP or PLIN3/TIP47 in yeast 2-hybrid analyses,7 mapping molecular differences between TPD52 and TPD52L1 may explain why TPD52 (and possibly TPD52L2) associates with LDs. Both TPD52 and TPD52L2 were identified as cholesterol-interacting proteins in HeLa cells, with both selectively binding a trans-sterol probe vs. a non-steroidal neutral lipid probe.41 Furthermore, both TPD52 and TPD52L2 have been reported to bind the phosphoinositide PI(3,4,5)P3,42 raising the possibility that a subset of TPD52-like proteins may bind lipid species directly.

Recent studies of protein binding to LDs have suggested a protein crowding model, where space is created as LDs expand, and proteins are crowded off as LDs shrink.43 If TPD52 binds LDs directly or indirectly, increased detection of TPD52 at the LD surface under conditions where droplets are expanding would be in accordance with a crowding model (Fig. 2).43 TPD52 binding to PLIN proteins would also be expected to result in increased TPD52 detection at the LD under...
conditions requiring lipid storage. However, the fact that TPD52 has been only infrequently identified as a component of the LD proteome agrees with only a small proportion of TPD52 being detected at LDs using fractionation studies, in marked contrast to Plin2/Adrp. This disparity between the relative enrichment of Plin2/Adrp and TPD52 at LDs argues against PLIN binding being the major determinant of TPD52 localization at the LD.

**TPD52 functions at the lipid droplet**

The actual function of TPD52 at the LD is also a matter of conjecture. Detection of TPD52 at LDs significantly increased in TPD52-expressing 3T3 cells treated with oleic acid, which could reflect active TPD52 recruitment in response to increased storage requirements (Fig. 2), but could also more passively reflect increased LD numbers. The relatively small amount of TPD52 detected within the LD fraction of oleic acid-treated cells suggests a transient association, consistent with TPD52 trafficking other proteins to/from the LD surface (Fig. 2). TPD52 could also compete within other LD proteins for binding, and thereby regulate the local abundance of other positive or negative regulators of lipid storage (Fig. 2).

In addition to the widely recognized differences in LD functions in different tissues, there are also multiple LD sub-types. For example, Wifling et al. identified LD populations that either remained static or expanded in response to oleic acid supplementation. Furthermore, the expanding LD sub-population was characterized by glycerol-3-phosphate acyltransferase-4 co-localization, a rate limiting enzyme in triglyceride synthesis. Infrequent detection of TPD52 within the LD proteome may also reflect that TPD52 associates with a subset of LDs within the cell, although a detailed analysis of TPD52 detection at LDs according to both size and responses to lipid loading will be required to determine whether this is true. As TPD52 expression in 3T3 cells was also associated with increased lipogenesis, TPD52 could directly facilitate triglyceride synthesis, either by trafficking and/or tethering components of the synthetic machinery to the LD surface, and/or by regulating the expression or activity of specific enzymes.

Figure 2. Possible mechanisms by which TPD52 might promote lipid storage in LDs, considering the predicted low abundance of TPD52 at the LD. TPD52 (shown in red) may traffic positive lipid storage regulators (shown in green) to the LD (1), and then may transiently bind the LD (2), prior to TPD52 release (3). TPD52 may (also) be recruited to bind and thereby remove negative storage regulators (shown in blue) from the LD surface (4). When bound to the LD, either in association with a positive storage regulator (5) or directly (6), TPD52 may also reduce the binding of negative storage regulators (7) by increasing local protein crowding.
Lipid droplets and cancer

Finally, Kamili et al.\textsuperscript{7} also discussed the possible relevance of TPD52’s function to cancer. A number of cancer-related phenotypes have been ascribed to TPD52, including increased cell proliferation and invasive capacity and reduced apoptosis and DNA repair capacity.\textsuperscript{10} There is therefore no shortage of explanations for why cancer cells might benefit from TPD52 overexpression, but these explanations are relatively generic and would not be expected to favor one cancer cell type over others. Where knock-down studies have been conducted in different breast cell lines, reduced TPD52 expression commonly reduced the survival of ERBB2-positive breast cancer cell lines,\textsuperscript{21,22} suggesting a particular function in this cell type. ERBB2-positive breast cancer cell lines are characterized by increased LDs,\textsuperscript{21} and ERBB2-positive breast cancers more frequently express proteins associated with lipogenesis or lipid storage.\textsuperscript{48} Increased TPD52 expression could therefore favor the survival of cell lines with increased lipogenic activity by contributing to the increased lipid storage capacity that these cells require.\textsuperscript{7} Again, further research will be necessary to determine whether there is an association between TPD52 expression and abundant LDs in cancer cell lines and the consequences of TPD52 knock-down in cancer cell lines with scarce versus abundant LDs.

Concluding remarks

Proteomic and RNAi screening approaches have collectively identified many LD-associated and -regulatory proteins.\textsuperscript{5,6,14-18,32-27,43} Although TPD52 did not emerge as a strong candidate from these collective studies, the direct expression of TPD52 in cultured cells resulted in significantly increased LD numbers.\textsuperscript{7} This finding suggests that there may be other important regulators of LDs identified through RNAi screening and proteomics approaches that are yet to be prioritised for further study.

As demonstrated by Kamili et al.,\textsuperscript{7} the direct testing of proteins that are infrequently but nonetheless recurrently identified by screening studies may identify bona fide LD regulators. While the analysis of possibly lower-abundance or itinerant LD proteins is likely to be more technically challenging, such proteins may provide a means to explore functional differences between LD subtypes and to subsequently exploit such differences for therapy. Understanding how proteins such as TPD52 “drop in” on the LD may therefore provide unexpected insights into the functions of other LD-associated proteins and how many LD regulators exert their functions from a distance within the cell.\textsuperscript{6}

Abbreviations

| LD | lipid droplet |
| PLIN | perilipin |
| TPD52 | tumor protein D52 |

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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