THROMBOSIS AND HEMOSTASIS

Factor XIII-A transglutaminase acts as a switch between preadipocyte proliferation and differentiation

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Key Points

- Preadipocytes produce factor XIII-A, which acts as a negative regulator of adipogenesis by increasing plasma fibronectin matrix assembly.
- Factor XIII-A and plasma fibronectin matrix promote preadipocyte proliferation and proproliferative effects of insulin.

Introduction

Obesity, which is characterized by abnormally high fat accumulation in adipose tissue and other organs, has a heritability range of 65% to 80%1,2 and is a risk factor for thrombosis and many severe chronic illnesses, including type 2 diabetes, coronary heart disease, arthritis, and cancer.3,4 Obesity is also associated with the hypercoagulable state caused by increased production of liver-derived clotting factors occurring as a reaction to increased circulating lipids and inflammatory cytokines caused by dysfunctioning adipose tissue.5-9 A recent genome-wide screen study examined gene expression changes linked to body mass index (BMI) from white adipose tissue (WAT) of monozygotic twin pairs discordant in BMI to seek potentially causative genes (vs index (BMI) from white adipose tissue (WAT) of monozygotic twin pairs discordant in BMI to seek potentially causative genes (vs index (BMI) from white adipose tissue (WAT) of monozygotic twin pairs discordant in BMI to seek potentially causative genes (vs index (BMI) from white adipose tissue (WAT) of monozygotic twin pairs discordant in BMI to seek potentially causative genes (vs index (BMI) from white adipose tissue (WAT) of monozygotic twin pairs discordant in BMI to seek potentially causative genes (vs


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bond (isopeptide crosslink/bond) that can induce the formation of multimeric protein networks and change the conformation, structure, solubility, biochemical stability, and cell-adhesion properties of substrate proteins.23-29 In addition to fibrin, a major extracellular substrate for FXIII-A is fibronectin (FN).30 FN is an extracellular glycoprotein capable of regulating various cellular functions, including proliferation and differentiation.31-33 FN is found in human and mouse WAT and preadipocyte cultures,34-36 where its role is associated with inhibition of adipogenesis.35,36 In the physiological setting, FN exists as 2 pools: as cellular FN (cFN) synthesized by tissue-resident cells and as plasma FN (pFN) produced by the liver.31 pFN has recently been shown to accumulate from the circulation into several tissues (liver, brain, testis, heart, lungs, and bone),37,38 and to contribute to the majority of the FN extracellular matrix associated with several cell types.16,31-33 FXIII-A has been shown to increase FN matrix accumulation in fibroblasts.39

Given the association between the FXIII-A and obesity and its potential presence in WAT, our aim here was to explore the role of TG activity and FXIII-A in adipogenesis. Here, we provide the first report demonstrating that differentiating preadipocytes have abundant TG activity that derives from FXIII-A. Our studies using 3T3-L1 preadipocytes as well as normal and F13a1-deficient mouse embryonic fibroblasts (MEFs) show that FXIII-A is located on the cell surface, where it exerts its effects via promoting soluble pFN assembly into extracellular matrix of preadipocytes. This maintains focal adhesions, promotes proliferation, and potentiates proproliferative effects of insulin (INS) while acting as an antagonist for adipocyte differentiation and lipid accumulation. Our work suggests a novel function for FXIII-A and circulating pFN in energy metabolism.

### Materials and methods

#### Animals

F13a1−/− mice were a generous gift from Dr Gerhard Dickneite (CSL Behring GmbH).40 Wild type mice (CBA/CaJ) were purchased from Jackson Laboratories (Bar Harbor, ME). Mice were kept under a normal diurnal cycle in a temperature-controlled room and fed with standard chow. Animal procedures (WAT extraction and MEFs isolation) and study protocols were approved by the McGill University Animal Care Committee.

#### Preadipocyte cell culture, differentiation, and Oil Red O staining

3T3-L1 cells (ATCC, Manassas, VA) were maintained in Dulbecco’s modified Eagle medium containing 10% calf serum, 100 U/mL penicillin G, and 100 µg/mL streptomycin. Differentiation into adipocytes was induced 2 days postconfluence with 10% fetal bovine serum (FBS), 1 µM dexamethasone (DEX), 0.5 mM isobutyl-1-methylxanthine (IBMX), and 1 µg/mL INS and is
referred to as differentiation media (DM). After 2 days, the DM was replaced with maintenance medium, which includes 10% FBS and 1 μg/mL INS. After 2 days, the maintenance medium was replaced with medium containing 10% FBS, with the endpoint of the experiment being day 8. On day 8, intracellular triglycerides were stained by Oil Red O and quantified; cells were counterstained with hematoxylin and photographed with a light microscope as previously described.41 Treatments included NC9 and NC10 (20-40 μM), PI3-kinase inhibitor LY294002 (10 μM), biotin-F11 (DQMMLPWPAVAL) and biotin-F11QN (DNMMLPWPAVAL) peptides (50 μM), picropodophyllin (10 μM), and hydroxy-2-naphthalenylmethylphosphonic acid trisacetoxymethyl ester (10 μM).

Cell proliferation assay

Proliferation experiments were done in 96-well plates or in 60-mm plates. 3T3-L1 cells or MEFs were serum-starved for 20 hours, after which 3 x 10^5 cells/mL cells were plated and stimulated with the indicated media for 24 hours followed by analysis using the 3-(4,5-dimethylthiazol-2-yl)-2,5-dimethyltetrazolium bromide; 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide assay (thiazolyl blue tetrazolium bromide) as previously described.19 Complete “Materials and methods” are available in supplemental Material on the Blood Web site.

Results

TG activity is present in mouse WAT, differentiating 3T3-L1 preadipocytes, and arises from FXIII-A.

To investigate the role of TG activity in adipogenesis, we first examined the presence of γ-glutamyl-ε-lysyl crosslinks (isopeptide bonds), indicative of TG activity, in mouse WAT. Immunohistochemistry demonstrated an abundance of isopeptide bonds at the periphery of adipocytes and adipose tissue stroma (Figure 1A). Assessment of TG activity using the 5-(biotinamido)pentylamine (BPA)
incorporation assay in extracts of different mouse WAT deposits showed that TG activity was present in epididymal, mesenteric, perirenal/retroperitoneal, inguinal, and subcutaneous WAT deposits in vivo (Figure 1B). Messenger RNA (mRNA) analysis of TG family members TG1–TG7 and FXIII-A in mouse WAT and in the 3T3-L1 cell line showed only Tgm2 and F13a1 expression (Figure 1C). Both enzymes were also detected by whole-mount immunofluorescence microscopy of mouse WAT (Figure 1D). 3T3-L1 cell line is commonly used to study adipogenesis in vitro; this cell line is derived from MEF that can differentiate into lipid-storing adipocytes upon stimulation with DM (containing INS, IBMX, and DEX). Analyses of TG expression during adipocyte differentiation of 3T3-L1 cells showed that Tgm2 mRNA levels did not change during differentiation, whereas F13a1 mRNA responded to DM by an initial decrease at day 1, followed by an increase at day 2, and finally gradually decreasing as the cells began to accumulate lipids (Figure 1E).

To determine whether TG activity was present during adipocyte differentiation, we assessed TG activity in situ by using BPA incorporation assay in differentiating 3T3-L1 cells treated with DM. A dramatic and significant induction of TG activity on days 1 and 2 was observed (8-fold and 10-fold increases, respectively, compared with day 0), and this was followed by a gradual decrease in activity as the cells matured into adipocytes (Figure 2A). To examine which of the 2 TGs were active during cell differentiation, NC9, a TG inhibitor containing a dansyl probe, was used to detect activity. NC9 incorporates irreversibly into active TG enzymes, including TG2 and FXIII-A. Here, we further demonstrate that NC9 incorporates into thrombin activated FXIII-A in vitro (supplemental Figure 1A–B), but not into nonactivated FXIII-A as shown by dansyl detection after western blotting (WB) (supplemental Figure 1B). Immunofluorescence staining of cells treated with NC9 for dansyl shows colocalization with FXIII-A at the cell periphery (Figure 2B). No colocalization with TG2 was observed in the cells (supplemental Figure 2). This suggests that preadipocyte TG activity arises from FXIII-A and that TG2 may not be active as a TG or not in its open active conformation in these cells. WB detection of dansyl in cell-surface preparations of preadipocytes showed strong dansyl incorporation, mostly into a protein band ~150 kDa and in lesser extend into a protein band between 50 and 75 kDa (Figure 2C). Immunoprecipitation of NC9 dansyl with dansyl antibody and detection with FXIII-A antibody demonstrated that the high-molecular weight (HMW) protein is FXIII-A (Figure 2D). HMW FXIII-A was also detected in 3T3-L1 extracts and MEFs, where it was induced upon differentiation treatment that also induced TG activity (Figure 2E). Because neither of the observed MWs correspond to the MOM of circulating FXIII-A monomer (83 kDa), we used 2 anti-human FXIII-A antibodies (A-4 and ab976362) to detect mouse preadipocyte FXIII-A together with human FXIII-A as a positive control. Supplemental Figure 1A shows Coomassie Blue staining of the nonactivated and thrombin-activated human FXIII, which runs at ~75 kDa. A HMW band was seen in the gels ~150 kDa upon thrombin activation and in the WBs using the 2 anti-human FXIII-A antibodies (Figure 2F–G). This HMW FXIII-A band is likely a dimer. Comparing human FXIII-A to mouse platelet-rich plasma and 3T3-L1 extracts showed that the mouse preparations had a FXIII-A protein of smaller MW than human FXIII-A (Figure 2F).

Another human FXIII-A antibody (Figure 2G) detected 2 FXIII-A bands in mouse platelet-rich plasma at 75 kDa and between 50 and 75 kDa, strongly suggesting that mouse platelets also have a smaller MW FXIII-A. Incubation of mouse platelet extracts and mouse plasma with NC9 in vitro showed its clear incorporation into a band between 50 and 75 kDa that corresponds to the smaller monomer FXIII-A found in preadipocytes (Figure 2H). The fact that ultimately 2 FXIII-A forms are detected in platelets suggests that the smaller form may be proteolytically cleaved from the full-length FXIII-A. Collectively, these results suggest that preadipocyte FXIII-A may be a cleaved form that complexes/dimerizes and gets activated at the cell surface of preadipocytes.

**FXIII-A acts as an antagonist for adipogenesis**

Given the high level of FXIII-A activity during adipocyte differentiation, we asked if the activity is required for cell differentiation. As

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**Figure 3. Inhibition of FXIII-A TG activity increases adipocyte differentiation and lipid accumulation.** (A) Inhibition of TG activity with the irreversible TG inhibitor NC9 increases lipid accumulation in a concentration-dependent manner as assessed by quantification of Oil Red O staining of 3T3-L1 cultures on day 8 of differentiation. Images show the increased size of lipid droplets in Oil Red O-stained cells. (B) Inhibition of TG activity during different stages of 3T3-L1 culture shows that TG activity has its most prominent inhibitory effect on lipid accumulation when given during days 0 to 4. (C–D) WB analysis and quantification of PPARγ expression (normalized to actin) during adipocyte differentiation shows increased expression (and thus accelerated differentiation) of NC9-treated cells. (E–F) WB analysis and quantification of Akt phosphorylation at Ser473 show that inhibition of TG activity significantly increases Akt activation. (G) The PI3K pathway inhibitor LY294002 used from day 0 to 4 reversed the NC9-mediated increase in adipogenesis; the graph shows quantification of Oil Red staining of the cultures on day 8. (H) Inhibition of TG activity with NC9 between days 0 and 4 can function in a similar manner as insulin (INS) in DM to promote preadipocyte differentiation; the graph shows quantification of Oil Red O-stained cultures on day 8. All error bars represent SEM (n = 3). *P < .05; **P < .01; ***P < .001.
shown in Figure 3A, NC9 significantly and in a concentration-dependent manner increased lipid accumulation and lipid droplet size in adipocytes. Control compound NC10, lacking the warhead acryloyl group, did not have an effect on adipogenesis (supplemental Figure 3). NC9 was most efficient in promoting lipid accumulation when given to 3T3-L1 cells between days 0 and 4, which enhanced lipid accumulation to the same extent as a full 8-day treatment. A significant increase in lipid accumulation was also seen when the inhibitor was given between days 0 and 2, and a decrease in lipid accumulation was observed in treatments occurring during days 4 to 8 (Figure 3B). Similar results were observed with MEFs, whose differentiation into adipocytes was promoted by NC9 in both a dose- and time-dependent manner (supplemental Figure 4). WB analysis and quantification of the adipogenic transcription factor peroxisome proliferator-activated receptor (PPARγ) showed a significant increase in NC9-treated cells (1.5-fold) compared with the control (Figure 3C-D). Analysis of phosphatidylinositol 3-kinase (PI3K)/Akt signaling, the main regulator of PPARγ expression, showed significant changes: (1) Akt phosphorylation was increased (1.5-fold) on day 1 following NC9 treatment (Figure 3E-F), and (2) LY294002, a PI3K inhibitor, reversed the increase in differentiation caused by NC9 (Figure 3G). Because inhibition of FXIII-A activity stimulated PI3K/Akt signaling, we differentiated cells with partial hormonal stimulation (DEX and IBMX only, no INS) in the presence and absence of NC9. Remarkably, inhibition of FXIII-A activity induced differentiation at a level similar to INS stimulation (Figure 3H). Collectively, these data strongly suggest that preadipocyte FXIII-A acts as an antagonist during the early phase of adipogenesis. No FXIII-A was detected in FBS used in the preadipocyte cultures (supplemental Figure 5), and thus its contribution to the observed effects can be excluded.

**FXIII-A activity promotes actin dynamics and focal adhesion formation in preadipocytes via crosslinking FN**

In the search of mechanisms for how preadipocyte FXIII-A inhibits adipogenesis and the PI3K/Akt pathway, we considered that TG activity has been linked with cell-matrix interactions and that during adipocyte differentiation, preadipocytes undergo a major morphological change where the transition from fibroblast-like (preadipocytes) to rounded (adipocyte) cells is associated with extensive cytoskeletal and matrix remodeling. An examination of cytoskeletal dynamics in NC9-treated preadipocytes plated on FN under serum-free conditions showed that the inhibitor dramatically reduced actin stress fiber formation and increased cortical actin assembly compared with control cells. This was also associated with reduced focal adhesion assembly as seen by a lack of vinculin colocalization with actin stress fibers (Figure 4A). WB analysis of subcellular fractions of these cells showed redistribution of vinculin from the cytoskeleton to the cytosol upon NC9 treatment (supplemental Figure 6A). Furthermore, NC9 also reduced preadipocyte adhesion (supplemental Figure 6B) and ROCK kinase activity (supplemental Figure 6C), necessary for the maintenance of actin stress fibers and focal adhesions. The dansyl group of NC9 was detected on the cell periphery of preadipocytes by immunofluorescence microscopy, which was associated with a reduction of actin stress fibers and rounding of the cells (Figure 4B). These results show that FXIII-A on the cell surface of preadipocytes promotes cell adhesion, actin stress fiber formation, and focal adhesion assembly. To examine FXIII-A substrates in these cultures, we conducted in situ labeling of preadipocytes with monodansylcadaverine (MDC), which incorporates covalently into TG-reactive Q residues of TG substrate proteins. MDC...
was found in protein(s) having an MW of 250 kDa (Figure 4C), which corresponds to FN (monomer). FN is one of the major extracellular FXIII-A substrates also linked to cytoskeletal dynamics.54-56 To examine whether the FXIII-A activity crosslinks (and labels) extracellular FN in preadipocyte cultures, MDC-labeled cells were immunoprecipitated using dansyl antibody and detected with FN antibody; this showed that the coated FN is a TG substrate in these cultures (Figure 4C). The effect of NC9 on FN labeling in the cultures was examined by an in situ TG activity assay, where BPA incorporation by the cells onto coated FN was quantified.57 NC9 decreased the amount of BPA incorporated into FN outside the cells by 50% compared with the DM-treated preadipocyte control (Figure 4D). In summary, these results confirmed that FXIII-A activity is predominantly found at the cell surface of preadipocytes and that FN is a major crosslinking substrate in the extracellular matrix.

FXIII-A activity is required for pFN matrix assembly and preadipocyte proliferation

We next investigated the function of FXIII-A activity with regards to the role of FN in preadipocytes. Enzyme-linked immunosorbent assay analysis of FN levels in cell layers showed increased levels of FN associated with initiation of differentiation, reaching a maximum by day 2; by day 4, FN levels decreased to the day 0 level (Figure 5A). This pattern was similar to the pattern of TG activity in the cultures as shown in Figure 2A. FN can be assembled into the cell layers as extracellular matrix from 2 pools of FN: from circulating pFN made by hepatocytes in liver (present in the serum used in cell cultures) and from cFN synthesized by tissue-resident cells.31,37 Both forms can be found as deoxycholate (DOC)-soluble and DOC-insoluble matrix. To investigate whether both pFN and cFN were substrates for FXIII-A activity, cell cultures were labeled with BPA, and the labeled material was affinity purified and detected with FN antibody (detects all FN) and extra domain A (EDA)-FN (cFN) antibody. Detection in this way showed labeling of only total FN but not cFN (EDA-FN), suggesting that only pFN is a substrate for TG activity (Figure 5B). To further confirm that FXIII-A in preadipocytes crosslinks pFN, we used a FXIII-A-specific substrate peptide, bF11, which is a biotinylated peptide containing a reactive glutamine (Q) residue58; this peptide is capable of incorporating into substrates only when FXIII-A is active. Cells were labeled with bF11 or control F11QN (where the Q is replaced by asparagine [N]) for 24 hours and pulled down with Neutravidin beads. WB showed clear detection of total FN with bF11 but not with control F11QN. NC9 blocked FN labeling by bF11 (Figure 5C). To further demonstrate that FXIII-A promotes pFN incorporation into matrix in preadipocyte cultures, cells were given exogenous biotinylated pFN (bpFN) in a pulse/chase experiment. Fluorescence microscopy of the bpFN matrix network showed that FXIII-A inhibition decreased FN assembly in preadipocyte cultures (Figure 5D). Levels of bpFN were analyzed from media and DOC-soluble and DOC-insoluble extracts after 24-hour incubation. These data show (1) a significantly higher level of bpFN retained in the media in NC9-treated cultures (Figure 5E); (2) intracellular FN levels, analyzed from trypsinized cells, which showed no change upon NC9 treatment, demonstrating that FN production was neither increased or decreased (Figure 5F); and (3) significantly lower bpFN levels in both DOC-soluble and DOC-insoluble matrix by NC9 treatment (Figure 5G-H). These data suggest that FXIII-A activity on the preadipocyte surface is specifically directed toward assembling a soluble form of pFN into preadipocyte extracellular matrix. Adding recombinant, soluble FXIII-A (activated) to the 3T3-L1 cultures along with bpFN did not result in
organized fibrillogenesis but rather aggregated bpFN, resulting in an increase in lipid accumulation (data not shown). This suggests that soluble FXIII-A may not promote bpFN fibrillogenesis in preadipocyte cultures.

After initiation of differentiation, 3T3-L1 cells undergo mitotic clonal expansion for 48 to 72 hours, which coincides with the increased FXIII-A activity (Figure 2A) and increased levels of FN in cell layers. Thus, we hypothesized that FXIII-A activity regulates pFN matrix assembly to promote preadipocyte proliferation. Proliferation was assessed by plating cells under serum-free conditions on pFN-coated plates or by supplementing the media with pFN. Proliferation assays showed that both ways of exposing the cells to pFN increased preadipocyte proliferation in a concentration-dependent manner (Figure 6A). A blocking antibody against EDA-FN further increased preadipocyte proliferation, suggesting that pFN and EDA-FN may have opposing functions in preadipocytes (Figure 6B). Cell proliferation can be induced by FN and soluble mitogens such as INS.\(^{59}\) Because INS was a component of the DM for preadipocytes, we examined the combined effects of INS and pFN on preadipocyte proliferation. The analyses showed a significant fourfold increase in proliferation by INS-pFN treatment compared with pFN treatment alone, demonstrating a synergistic effect. The proproliferative effect was dependent on FXIII-A activity, because NC9 significantly attenuated these effects for both the pFN treatment alone and the INS-pFN treatment combination (Figure 6C). It is known that INS mediates cell proliferation via activating the mitogen-activated protein kinase/extracellular signal-regulated kinase (Erk) pathway.\(^{60}\) Analysis of Erk phosphorylation levels in pFN- and INS-treated preadipocytes showed that NC9 decreased the sustained Erk phosphorylation in these cells in both serum and serum-free conditions (supplemental Figure 7). To examine whether INS effects are mediated through the INS-like growth factor receptor or through the INS receptor, we inhibited both receptors and assessed cell proliferation. As seen in Figure 6D, only the INS receptor inhibitor HNMPA-(AM)3 was able to decrease the combined proproliferative effects of pFN and INS. Inhibition of INS-like growth factor receptor with its specific inhibitor PPP had an opposite effect and promoted cell proliferation. These results indicate that FXIII-A–mediated assembly of pFN is required for proliferation of preadipocytes and can potentiate the proproliferative effects of INS.

**F13a1\(^{-/-}\) MEFs show reduced cell adhesion and proliferation and increased adipogenesis**

To confirm the role of FXIII-A in adipocyte function, we examined the ability of F13a1\(^{-/-}\) MEFs to proliferate and differentiate into adipocytes. Compared with F13a1\(^{+/+}\) cells, F13a1\(^{-/-}\) MEFs exhibited a 30% increase in lipid accumulation (Figure 7A; supplemental Figure 8A) and decreased cell adhesion to pFN (supplemental Figure 8B). mRNA expression of F13a1 and Tgm2 were not altered during differentiation of F13a1\(^{-/-}\) MEFs (supplemental Figure 8C). F13a1\(^{-/-}\) MEFs also showed a significant decrease in their ability to proliferate and a decreased response to the proliferative effects of exogenous pFN supplemented into the serum-free media. The F13a1\(^{-/-}\) MEFs also showed an overall reduced proliferative response to INS with or without exogenous pFN (Figure 7B). Fluorescence microscopy of bpFN in MEFs showed that F13a1\(^{-/-}\) cells assembled bpFN poorly into the fibrillar matrix compared with F13a1\(^{+/+}\) cells (Figure 7C). These data show that pFN constitutes the majority of the total FN extracellular matrix in preadipocytes/MEFs and requires FXIII-A for its assembly.

**Discussion**

Recent genome-wide association studies of human WAT identified F13A1 as a potentially causative gene for obesity,\(^{10}\) suggesting that FXIII-A may be linked to adipose tissue function. In our study, we provide the first set of evidence showing that WAT has abundant FXIII-A activity and how FXIII-A can be linked to adipogenesis. FXIII-A enzyme was localized to the preadipocyte surface, where it assisted in assembling pFN into the matrix to promote cell proliferation and to potentiate the proproliferative effects of INS. This antagonized the prodifferentiating effects of INS on preadipocytes.
FXIII-A, jointly with pFN, maintained preadipocytes in an undifferentiated state by modulating cytoskeletal dynamics. Thus, we conclude that FXIII-A acts as a negative regulator of adipogenesis. Our study also demonstrated that preadipocytes express TG2; however, TG activity probe and inhibitor NC9 did not covalently incorporate into TG2 based on immuno-fluorescence data, indicating that it is not active as a TG enzyme. However, because its function has been strongly linked to cytoskeletal dynamics, it may also contribute to maintenance of the cytoskeleton of preadipocytes/adipocytes via a mechanism that does not involve its TG activity.

Preadipocyte differentiation into lipid-accumulating mature adipocytes is part of normal adipose tissue function and is critical for storage and elimination of lipids from the circulation. Preadipocyte proliferation is required for adipose tissue expansion to accommodate the increased requirement for energy storage in obesity. Failure to accumulate lipids or to expand adipocyte tissue results in increased circulating fatty acids and their ectopic storage in nonmetabolic tissues, which is a major contributor to the development of INS resistance. Our results show that FXIII-A, jointly with pFN, increases preadipocyte proliferation, but inhibits lipid accumulation.

The role of FN as a negative regulator of adipogenesis in vitro has been demonstrated in mouse and human preadipocytes, where it inhibits lipid accumulation by blocking the morphological and cytoskeletal changes necessary for lipid accumulation. Furthermore, although FN matrix is clearly an important component of WAT and preadipocyte cultures, its actual function has remained elusive. Our work shows that preadipocytes use pFN matrix for proliferation and that this matrix sensitizes the cells for the proproliferative effects of insulin. Vascularization of WAT is critical for adipose tissue expansion during increased need for energy storage; it is likely that pFN is one of the circulating factors that can regulate this tissue expansion.

The transition of preadipocytes from a proliferation phase to a differentiation phase is reflected by changes in cell morphology accompanied by major remodeling of extracellular matrix components. Wherein preadipocytes themselves regulate synthesis and degradation of collagen and laminin matrices; the pFN matrix accumulation appears to be regulated by the presence of FXIII-A in the cells.
pFN levels in preadipocyte cultures follow the pattern of F13a1 mRNA and enzyme activity, and a decrease in FN matrix was associated with decreased F13a1 mRNA levels by the cells. Thus, FXIII-A regulation in preadipocytes may be part of the transition between the proliferative and differentiation states (Figure 7D-E). The preadipocyte FXIII-A is found on the cell surface mostly as a complex form. Similar HMWF FXIII-A was found to form upon activation of human FXIII in vitro, suggesting that preadipocyte cell surface FXIII-A may be a covalent, active dimer. Whether this preadipocyte FXIII-A requires further proteolytic activation remains unknown; however, it is possible that the observed dimerization/complexation, together with increased Ca²⁺ levels, and binding to substrate in the extracellular space suffices to induce activity. Of interest is also the observation that preadipocytes produce mostly FXIII-A monomer of lower MW. This form is also found in platelets, which produce 2 forms as per to our antibody detection data. Thus, it is possible that this smaller monomer FXIII-A, in both platelets and adipocytes, is a proteolytically cleaved form of the full-length FXIII-A and that the cleavage process could be linked to mechanisms on how FXIII-A is anchored to the cell surface.

FXIII-A deficiency in humans results in a rare blood-clotting defect.⁷⁰ There are no reports of energy metabolism dysregulation or BMI-linked abnormalities in FXIII-A–deficient patients; however, circulating FXIII-A levels are increased in type 2 patients with diabetes.⁶⁴ Thus, it is possible that obesity-linked F13A1 SNPs in WAT discovered in the ENGAGE study¹⁰ may have effects on FXIII-A function only locally in adipose tissue while having no effects on coagulation or other cellular processes. Indeed, a specific regulation, modification, and function of FXIII-A in WAT is supported by the observation that the Finnish twins discordant in BMI and having altered FXIII-A expression in WAT have normal FXIII-A levels in blood.⁶ Similarly, the FXIII-A Val34Leu polymorphism, which results in increased enzyme activation, has a protective effect against coronary artery disease¹²,⁷² but is not linked to obesity.¹⁰ In conclusion, our study shows the presence and relevance of FXIII-A in adipose tissue and preadipocytes, suggesting a mechanism by which FXIII-A might be linked to obesity and weight gain. Eluciating the full metabolic phenotype of F13a1⁻/⁻ mice, and understanding how FXIII-A is modulated, processed, secreted, and anchored to the cell surface in adipocytes in the normal vs obese state, can provide valuable information about how to regulate adipose tissue health.

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Factor XIII-A transglutaminase acts as a switch between preadipocyte proliferation and differentiation

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