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Modification of the inflammatory mediator LRRFIP2 by the ubiquitin-like protein FAT10 inhibits its activity during cellular response to LPS

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ABSTRACT

FAT10 is a ubiquitin-like protein made of two tandem, head-to-tail, ubiquitin domains. It is known to covalently modify proteins in a mechanism similar, though not identical, to that of other ubiquitin-like proteins. The lack of known physiological substrates covalently conjugated by the protein made it difficult to unravel its biological functions. Here we identify two proteins that are covalently modified by FAT10, the inflammatory mediator LRRFIP2 and the endoplasmic reticulum membrane protein LULL1. LRRFIP2 is involved in NF-κB activation following stimulation of TLR4. It is recruited along with MYD88 to the cytosolic tail of the receptor, and by that mediates activation of the downstream signaling cascade. We show that FATylation of LRRFIP2 occurs on two distinct sites, each being modified by a single FAT10 moiety. Furthermore, the second modification is regulated by the first one. Importantly, FATylation of LRRFIP2 interferes with its recruitment to the membrane by translocating it to the cellular insoluble fraction, thus inhibiting NF-κB activation.

1. Introduction

FAT10 (HLA-F-Associated Transcript 10), also called UBD or diubiquitin, is a ubiquitin-like protein that was first identified in dendritic and mature B cells [1]. Basal expression of FAT10 is detected only in lymphoid organs [2,3], but carcinogenesis [2,4,5] or synergistic stimuli by the pro-inflammatory cytokines interferon γ (IFN γ) and tissue necrosis factor α (TNFα) [6,7] result in an up-regulation of its expression, suggesting that FAT10 plays a role in the signaling cascades of these cytokines.

FAT10 is an 18 kDa protein containing two tandem, head-to-tail, ubiquitin domains. Like ubiquitin, FAT10 displays a C-terminal di-glycine motif mediating its covalent binding to target proteins [8–10]. UBA6, an E1-like ubiquitin-activating enzyme, activates both ubiquitin and FAT10, but with a higher affinity towards FAT10 [11,12]. Unlike ubiquitin, which has a half-life of 9–10 h [13] and is degraded independently of further ubiquitination [8,14], FAT10 degradation occurs rapidly (hal-life of less than 1 h) and requires its polyubiquitination [8]. This rapid turnover (expression and degradation) probably reflects a tight regulation required under certain conditions for fast induction of FAT10 followed by effective degradation that turns off its activity.

Although FATylated species are readily detected along a wide range of molecular weights in whole cell extracts expressing WT FAT10 [8,9], only a handful of naturally occurring FAT10 targets have been identified thus far. One of them is p62; its FATylation results in its proteasomal degradation, though neither the mechanism of targeting of the protein to the proteasome, nor the biological significance of its FATylation have been unraveled [15]. Another identified substrate is p53. Its modification appears to regulate its transcriptional activity [16], suggesting that FATylation of proteins has a role in modulating their activity, additionally to serving as a degradation signal.

In our search for proteins that are modified by FATylation, we identified by mass spectrometry LRRFIP2 (Leucine-Rich Repeat Fli-I-Interacting Protein 2), a positive regulator of NF-κB activity in TLR4-mediated inflammatory response [17,18]. TLR signaling is vital for the innate immune response to infection. Stimulation with LPS initiates the activation of NF-κB by recruitment of LRRFIP2 to MYD88 (MYeloid Differentiation primary response gene 88), a common intracellular adaptor located downstream to TLRs. In addition to LRRFIP2, we identified LULL1 (TOR1AIP2 – TORsin 1A-Interacting Protein 2) as a FATylation substrate. LULL1 is an endoplasmic reticulum membrane protein, the function of which is largely unknown. The identification of LULL1 as a FATylation target attests to the validity of our detection method, though the current study focuses on the function of LRRFIP2 FATylation.

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2. Materials and methods

2.1. Cell culture and transfection

HEK-293 and HeLa cells were grown at 37 °C in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal calf serum, 0.2 mM L-glutamine, 10 U/ml penicillin and 10 μg/ml streptomycin. HEK-293-TLR4 (Toll-Like Receptor 4) cells (HEK-293/hTLR4A-MD2-CD14 line from Invivogen) were grown according to the manufacturer's instructions. All transfections were carried out using the standard calcium phosphate method.

2.2. Cell lysates for immunoprecipitation

Cell lysates from 100 mm dishes were prepared in RIPA containing protease inhibitors (Complete, Roche), and incubated for 20 h at 4 °C with 5 μl of immobilized anti-HA (clone 3F10, Roche). The beads were washed twice in 1 ml of RIPA and proteins eluted by boiling in sample buffer.

2.3. Cell fractionation

Cell pellets from a 100 mm dish were frozen in liquid nitrogen and resuspended in 200 μl HES buffer (20 mM HEPES pH 7.4, 1 mM EDTA, 250 mM Sucrose). Lysis was carried out by 40 strokes using a 25G needle. Nuclei and insoluble proteins were separated from the cytoplasm by centrifugation at 1000*g* for 3 min and then washed twice in 400 μl HES buffer. Cytoplasmic extracts were centrifuged for 2 h at 20,000*g* and the resulting pellet (membrane fraction) was boiled for 1 h in 100 μl of sample buffer. Nuclei were lysed in 100 μl of HES buffer containing 0.2% NP-40. Chromatin and insoluble proteins were separated from the nucleoplasmic extract by centrifugation at 20,000*g* for 30 min, and boiled in 100 μl of sample buffer.

2.4. Antibodies

Antibodies were directed against HA (clone 16B12, Covance), RGS-His (mouse anti-RGS(H)4 BSA-free, Qiagen) and Myc (clone 9E10, Santa Cruz) epitopes, Actin (clone C4, Millipore), α-tubulin (clone DM1A, Sigma), EGFR (1005, rabbit polyclonal, Santa Cruz), and ubiquityl-H2A (ubH2A, clone E6C5, Upstate-Millipore).

2.5. In gel proteolysis and mass spectrometry analysis

Cell lysates of HEK-293 cells transfected with pCS2-HA-FAT10-GG or -GV, along with pCS2-FLAG-UBA6, were subjected to immunoprecipitation using immobilized anti-HA. Immunoprecipitated proteins were resolved by electrophoresis followed by silver staining of the gel. Gel fragments containing the protein bands appearing selectively in the WT FAT10-expressing cells were excised and destained with 30 mM $C_6N_6FeK_3$ and 100 mM $Na_2S_2O_3$. Proteins were reduced with 2.8 mM DTT (60 °C, 30 min), modified with 8.8 mM iodoacetamide in 100 mM $(NH_4)HCO_3$ (30 min at room temperature in the dark) and digested (overnight, 37 °C) in 10% acetonitrile and 10 mM $(NH_4)HCO_3$ with modified trypsin (Promega). The tryptic peptides were desalted on a C18 trap column and resolved by reverse-phase chromatography on 0.075 × 200 mm fused silica capillaries (J&W) packed with Reprosil reversed phase material (Dr. Maisch; GmbH, Germany). The peptides were eluted with linear 45 min gradient of 7–40% followed by 8 min wash with 95% acetonitrile/0.1% formic acid in water at flow rates of 0.25 μl/min. Mass spectrometry was performed by an ion-trap mass spectrometer (OrbitrapXP, Thermo) in a positive mode using repetitively full MS scan followed by collision-induces dissociation (CID) of the 7 most dominant ion selected from the first MS scan. The mass spectrometry data were analyzed by the Bioworks 3.31 software using the Sequest search engine searching against the human section of the Uniprot database. Mass modifications for FATylation were added to the search.

2.6. NF-κB activation

HEK-293-TLR4 cells transfected with NF-κB and renilla luciferases reporter plasmids [23] were treated by 5 μg/ml lipopolysaccharide (LPS, Invivogen). Luciferase activity in cell lysates was measured and normalized for renilla activity using a dual luciferase assay system (Promega) and a Glomax 20/20 luminometer (Promega).

3. Results

3.1. Identification of LRRFIP2 and LULL1 as FATylation substrates

To identify FATylation substrates, we co-expressed HA-FAT10-GG or its FATylation-defective counterpart HA-FAT10-GV with UBA6 in HEK 293 cells. After HA immunoprecipitation, FATylated substrates and FAT10-associated proteins were resolved by SDS–PAGE and detected by silver staining. We detected two bands that were immunoprecipitated only from cells expressing WT but not mutant FAT10, and can therefore represent FATylated substrates. Following in gel tryptic digestion of these proteins, we identified by mass spectrometry peptides corresponding to FAT10 along with LRRFIP2 and LULL1 (Table 1). Since the position in the gel in which these proteins were identified was higher than their molecular mass, we assumed that they were indeed modified covalently by FAT10. Moreover, a putative FATylation site was identified at lysine 276 of LRRFIP2, as shown by the molecular weight shift of the peptide containing this lysine (Table 1). This shift is attributed to the C-terminal fragment of FAT10 (residues 152–165) attached to lysine 276 of LRRFIP2. To confirm that LRRFIP2 and LULL1 can be indeed FATylated, we cloned the cDNAs of LRRFIP2 (isoform 2) and LULL1 in the pCS2-His plasmid, and expressed them in the presence of HA-FAT10-GG or HA-FAT10-GV, and UBA6. Following HA immunoprecipitation, LRRFIP2 and LULL1 were detected by Western blot using an anti-His antibody. Both proteins were able to interact with HA-FAT10 independently of the integrity of its di-glycine motif. However, in the presence of HA-FAT10-GG, LRRFIP2 (Fig. 1A) and LULL1 (data not shown) were also detected at a molecular weight zone which is higher than their mass, strongly suggesting that these species represent the FAT10 covalently-modified proteins. The co-expressed control protein His-RFP did not interact with HA-FAT10 and was not detected in the immunoprecipitated material.

Table 1
Sequences of FAT10, LRRFIP2 and LULL1 peptides identified by mass spectrometry.

Protein	Sequence	Mass
LRRFIP2 (Isoform 2)	FSAEDEALSNIAR	1422.686
	NSASATTPLSGNSSR	1449.693
	AEQDITTLEQSISR	1550.797
	KLQLEEERQK (+1367,67)C	2771.401
LULL1 (TOR1A1P2)	ADAHLGSSSVALPK	1352.717
	VSPIQIDGAGR	1112.605
FAT10	TKVPVQDQVLLLGSK	1624.964
	SLSSYGIDKEK	1226.626
	VPVQDQVLLLGSK	1395.821
	VVKPSDEELPLFLVESGDEAK	2301.186
	SEEWDLM (+16)TFDANPYDSVK	2162.927

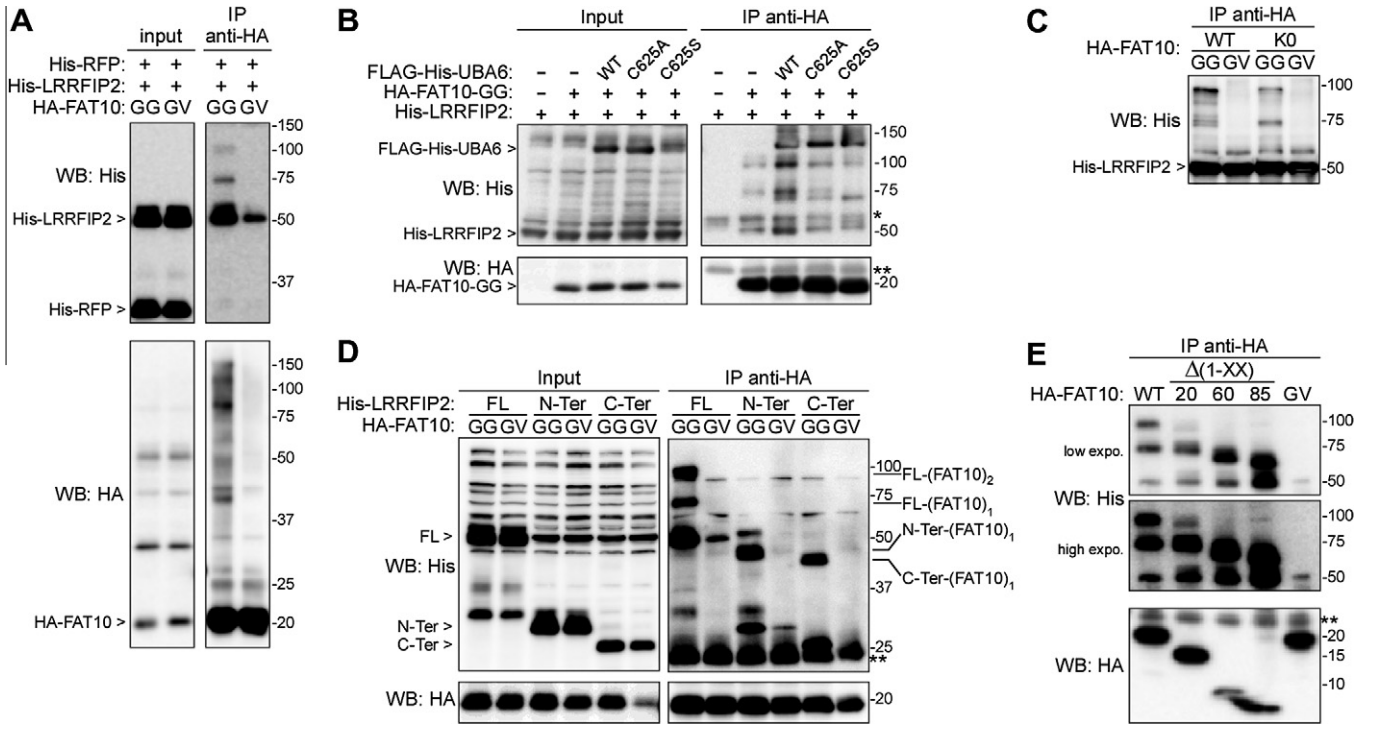

Fig. 1. LRRFIP2 is modified by two single moieties of FAT10 at two distinct sites. (A) HEK-293 cells were transfected with pCS2-FLAG-UBA6 and pCS2-HA-FAT10-GG or -GV (as indicated) along with pCS2-His-RFP and pCS2-His-LRRFIP2. After 48 h, cells were lysed and proteins were immunoprecipitated with immobilized anti-HA, resolved by SDS-PAGE, and detected following Western blot (WB) with the indicated antibodies as described under Section 2. Molecular weight markers are shown. (B) HEK-293 cells were transfected with pCS2-HA-FAT10-GG and pCS2-His-LRRFIP2 along with pCS2-FLAG-His-UBA6 or its catalytic mutants C625A or C625S as indicated. After 48 h, whole cell lysates were subjected to immunoprecipitation using immobilized anti-HA. Total and immunoprecipitated proteins were analyzed by SDS–PAGE and WB with the indicated antibodies. Molecular weight markers and immunoglobulin heavy (*) and light (**) chains are indicated. (C) HEK-293 cells were transfected with pCS2-His-LRRFIP2 and pCS2-FLAG-UBA6 along with different HA-FAT10-encoding plasmids as indicated. After 48 h, whole cell lysates were analyzed as in (A). (D) HEK-293 cells were transfected with pCS2-FLAG-UBA6 and pCS2-HA-FAT10-GG or -GV along with pCS2-His-LRRFIP2 (full length, FL), pCS2-His-LRRFIP2-NT (N-terminal half; 1–218) or -CT (C-terminal half; 219–423), as indicated. After 48 h, whole cell lysates were analyzed as in (A). Immunoglobulin light chains (**) and the different FATylation products of the WT and truncated species of His-LRRFIP2 are indicated. (E) HEK-293 cells were transfected with pCS2-FLAG-UBA6 and pCS2-His-LRRFIP2 along with pCS2-HA-FAT10-GG, pCS2-HA-FAT10(Δ1–20)-GG, pCS2-HA-FAT10(Δ1–60)-GG, pCS2-HA-FAT10(Δ1–85)-GG or pCS2-HA-FAT10-GV, as indicated. After 48 h, whole cell lysates were prepared and analyzed as in (A). Immunoglobulin light chains (**) are indicated.

3.2. LRRFIP2 is modified by two single FAT10 moieties

To confirm the activation role of UBA6, we co-expressed His-LRRFIP2 and HA-FAT10-GG in the presence of WT UBA6, or two catalytic mutants of the enzyme-C625A and C625S [12]. As shown in Fig. 1B, only WT UBA6 stimulated significantly the FATylation of His-LRRFIP2, demonstrating the requirement of the catalytic C625 for the activation of FAT10. To further characterize the FATylation reaction, we co-expressed His-LRRFIP2 and WT UBA6 along with HA-FAT10-GG or HA-FAT10-GV and their lysine-less counterparts (K0). WT and K0 variants of HA-FAT10 presented the same FATylation profile in which two FAT10 molecules were attached to His-LRRFIP2 (Fig. 1C), suggesting that LRRFIP2 is modified by two FAT10 moieties at distinct sites. Moreover, we showed that each half of LRRFIP2 is FATylated only once (Fig. 1D). An interesting finding involves the relationship between the two modifications of LRRFIP2, which appear to be linked and dependent on the structure of FAT10. Gradual truncation of FAT10 from its N-terminus inhibits progressively the second FATylation, suggesting that its N-terminal domain may serve as a sensor for the second FAT10 to modify LRRFIP2; while removal of the first 20 N-terminal residues affects the second FATylation only partially, truncation of longer segments abolishes the second modification completely (Fig. 1E). The first modification appears to be unaffected by the truncations, suggesting that the activation by UBA6 of the truncated FAT10 species proceeds efficiently.

3.3. FATylation of LRRFIP2 affects its subcellular localization

During activation of NF-κB by LPS, MYD88 and its co-activator LRRFIP2 are recruited to the cytosolic domain of TLR4 where they interact [17,18]. The isoform 2 of LRRFIP2 used in this study also interacts with MYD88 (Fig. S1). To investigate the effect of LRRFIP2 FATylation in this process, we studied its subcellular localization in the presence of HA-FAT10-GG, HA-FAT10-GV, or the control protein HA-GFP (Fig. 2). Cells were separated into membranes, cytosol and insoluble material (containing cytosolic aggregates and chromatin). Overexpression of HA-FAT10-GG, but not HA-FAT10-GV or HA-GFP, reduced the amount of His-LRRFIP2 in the membrane and cytosolic fractions, without affecting the localization of Myc-MYD88 or FLAG-His-UBA6 (Fig. 2A). Moreover, expression of HA-FAT10-GG increased the amount of LRRFIP2 in the insoluble fraction, where almost all FAT10 adducts of LRRFIP2 were localized (Fig. 2A). We have previously shown the tendency of FAT10 to precipitate in an insoluble form [8], and it is important to note that similar to the free protein, FAT10 conjugates also accumulate in the insoluble fraction (Fig. 2A; anti-HA WB).

It has been shown that phosphorylation of LRRFIP2 is critical for its function in NF-κB activation [18]. In the experiment presented in Fig. 2A, one can observe that LRRFIP2 appears as a doublet, and the localization of the upper band (assumingly the phosphorylated form of LRRFIP2) appears to be mostly affected by HA-FAT10. A treatment of the membrane and insoluble fractions with calf

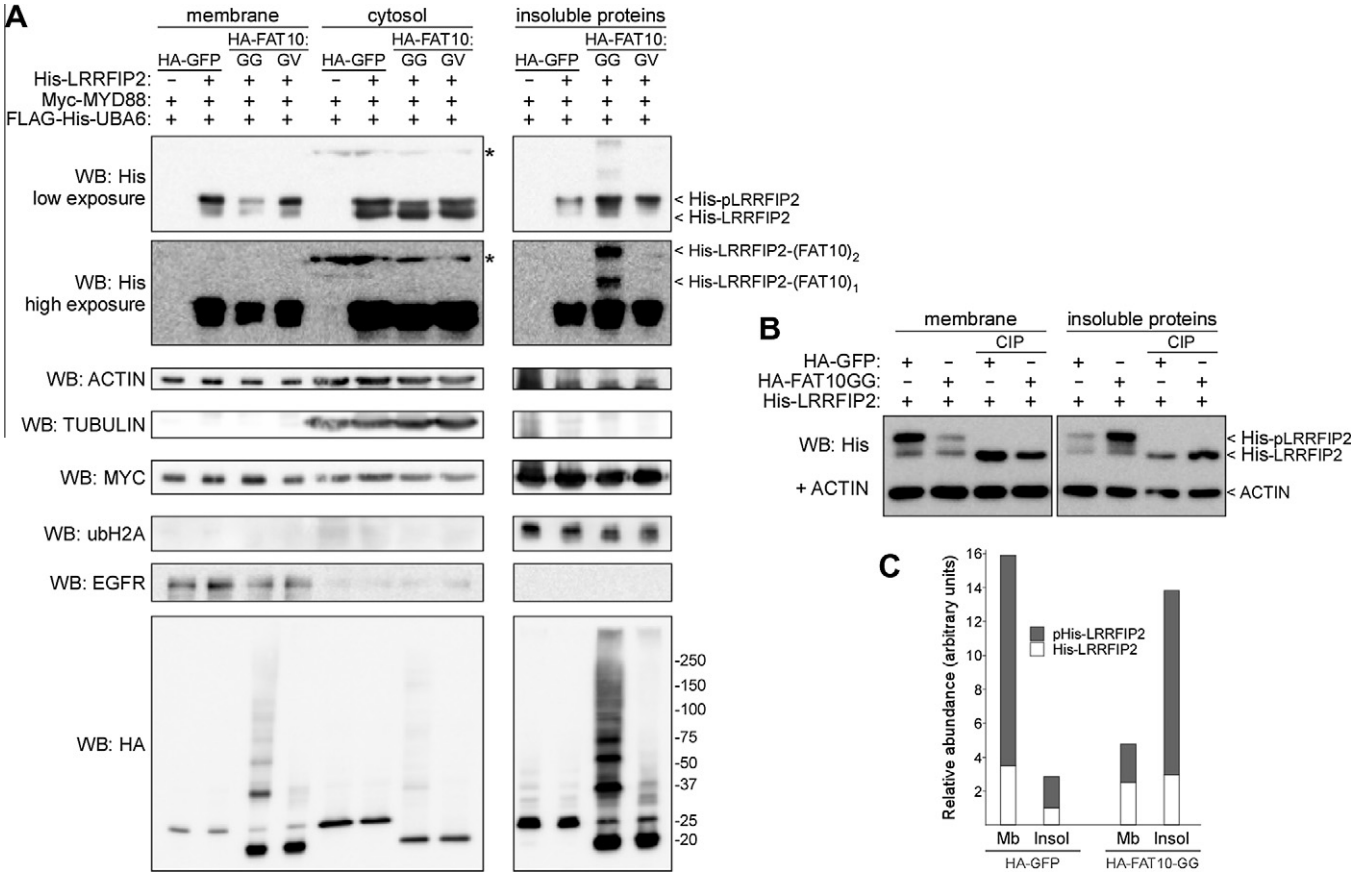

Fig. 2. Expression of FAT10 reduces the amount of LRRFIP2 at the membrane. (A) HEK-293 cells were transfected with empty pCS2 or pCS2-His-LRRFIP2, along with pCS2-Myc-MYD88, pCS2-FLAG-His-UBA6, and pCS2-HA-GFP or pCS2-HA-FAT10-GG or pCS2-HA-FAT10-GV, as indicated. After 48 h, cells were fractionated into membranes, cytosol and insoluble material as described under Section 2. Extracts were boiled in sample buffer, resolved by SDS–PAGE and analyzed by WB with the indicated antibodies. The position of FLAG-His-UBA6 is indicated (∗), as well as the position of the phosphorylated, non-phosphorylated, and mono- and biFATylated His-LRRFIP2. (B) Membrane and insoluble proteins extracts were prepared as described under (A) and were mock- or CIP-treated for 1 h at 37 °C and then denatured by boiling in sample buffer. Lysates were resolved by SDS–PAGE and analyzed by WB with anti-His and anti-actin antibodies. (C) Signals corresponding to phosphorylated and non-phosphorylated His-LRRFIP2 in (B) were quantified and normalized compared to the actin signal. Relative abundance of each of them is represented in arbitrary units.

intestine phosphatase (CIP) induced the disappearing of the upper band, confirming that it corresponds to the phosphorylated form of LRRFIP2 (Fig. 2B). Quantification of the LRRFIP2 band in the experiment shown in Fig. 2B reveals that expression of HA-FAT10-GG induces a 4-fold decrease in the amount of the protein in the membrane fraction accompanied by a parallel increase in its amount in the insoluble fraction (Fig. 2C). These changes were mainly due to variation in the amount of the phosphorylated form of LRRFIP2. We conclude that FATylation of LRRFIP2 alters the subcellular localization of its active form.

3.4. FAT10 and LRRFIP2 are co-localized in cytosolic aggregates

We showed in the experiment depicted in Fig. 2A (anti-HA WB) and in a previous study [8] that FAT10 has a tendency to aggregate. In order to visualize the cellular localization of FAT10 and LRRFIP2, we expressed both proteins fused to GFP and RFP, respectively (Fig. 3). When WT FAT10 was co-expressed with LRRFIP2, the two proteins were co-localized in dots, probably aggregates, in a significantly (2-fold) higher frequency compared to when mutant FAT10 was expressed (compare Fig. 3A, ii and iii– merge; quantification of data shown in Fig. 3B). This observation, along with the data shown in Fig. 2, suggests that the C-terminal di-glycine motif of FAT10 is crucial for its effect on the cellular localization of LRRFIP2. By promoting the translocation of LRRFIP2 into cytoplasmic aggregates, FAT10 might block its recruitment to the membrane and its subsequent role in NF-κB activation through TLR4 stimulation.

3.5. FAT10 inhibits activation of NF-κB after LPS stimulation

We have shown that FAT10 can be conjugated to LRRFIP2 and by that may interfere with its normal localization in the cell. Because LRRFIP2 is implicated in the activation of NF-κB after stimulation of TLR4 by LPS [17,18], we decided to test whether FATylation of LRRFIP2 alters activation of NF-κB. As can be seen in Fig. 4Ai, activation of NF-κB by LPS was significantly inhibited by HA-FAT10-GG but not by HA-FAT10-GV. It should be noted that the expression of the two FAT10 species was comparable in all cells tested (Fig. 4Aii). This suggests that FATylation of LRRFIP2 and possibly additional or other proteins negatively regulates LPS-mediated activation of NF-κB. The importance of LRRFIP2 fatylation in this process was confirmed by another experiment: as seen in Fig. 4Bi, LPS induces a strong association between LRRFIP2 and the cell membrane (compare lanes 1 and 4), which is strongly inhibited by WT but less so by mutant FAT10-GV (compare lanes 5–2 and 6–3). Quantification of these data is presented in Fig. 4Bii. Taken together, these results indicate that FATylation of LRRFIP2 modifies its subcellular localization, and via this mechanism, might negatively regulate NF-κB activation by LPS.

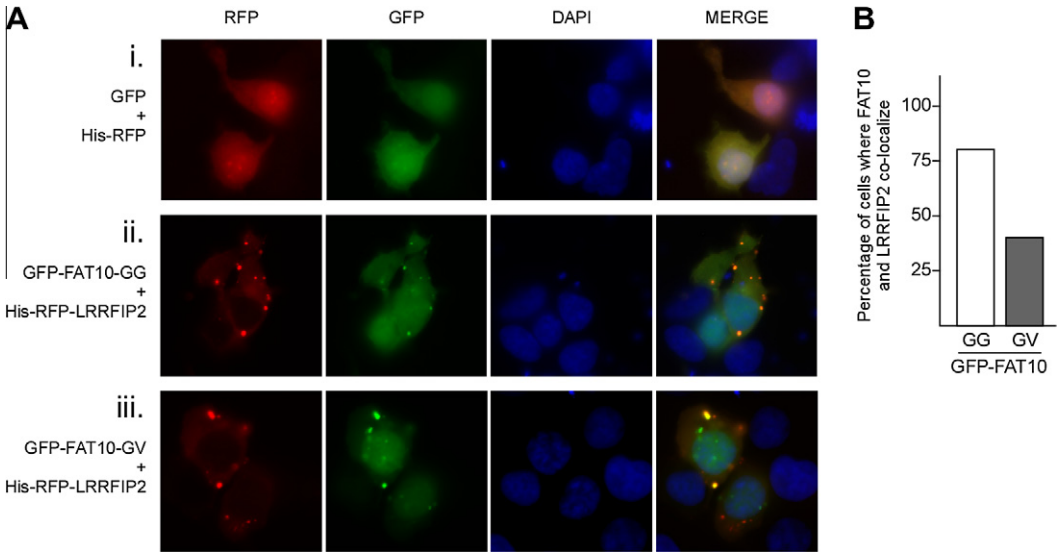

Fig. 3. FAT10 and LRRFIP2 are co-localized in cytosolic speckles. (A) HeLa cells were grown for one day on glass slides and then transfected with pCS2-His-RFP (i) or pCS2-His-RFP-LRRFIP2 (ii, iii) along with pCS2-GFP (i), pCS2-GFP-FAT10-GG (ii) or pCS2-GFP-FAT10-GV (iii). After 48 h, cells were fixed (4% paraformaldehyde in PBS), permeabilized (0.4% Triton X-100 in PBS), blocked (0.4% Tween20 and 5% BSA in PBS), and finally mounted using Vectashield with DAPI (Vector Laboratories Inc.). Fluorescent signals corresponding to RFP, GFP, DAPI and all three merged are shown. (B) Quantification of dots where LRRFIP2 co-localizes with either WT FAT10 or mutant FAT10-GV. Data were collected from 100 cells each expressing LRRFIP2 along with either WT FAT10 or mutant FAT10-GV.

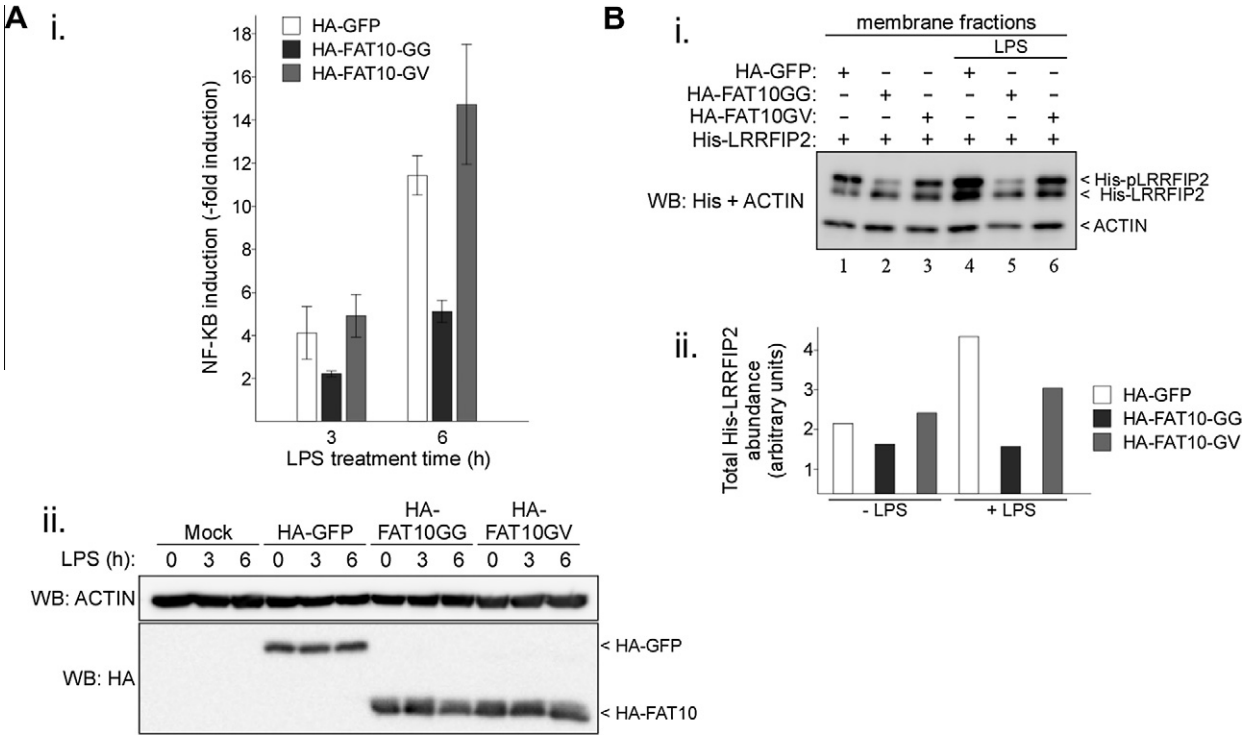

Fig. 4. FAT10 inhibits LRRFIP2 activity in LPS-mediated NF-κB activation. (A) HEK-293-TLR4 cells were transfected with cDNAs coding for NF-κB and Renilla reporter plasmids, along with pCS2-HA-GFP, pCS2-HA-FAT10-GG or pCS2-HA-FAT10-GV. After 40 h, cells were treated with LPS for 3 or 6 h. NF-κB activation was monitored and quantified in cell lysates (i) as described in Section 2. Whole cell extracts were analyzed by SDS–PAGE and WB using indicated antibodies (ii). (B) HEK-293-TLR4 cells were transfected with pCS2-His-LRRFIP2 along with pCS2-HA-GFP, pCS2-HA-FAT10-GG or pCS2-HA-FAT10-GV. After 44 h, cells were treated with LPS for 1 h. Membrane proteins were isolated as described under Section 2 and analyzed by SDS–PAGE and WB using anti-His and anti-actin antibodies (i). Signals corresponding to phosphorylated and non-phosphorylated His-LRRFIP2 were quantified, normalized compared to the actin signal, and represented in arbitrary units as the total amount of His-LRRFIP2 (phosphorylated and non-phosphorylated) at the membrane in the presence or absence of LPS stimulation (ii).

4. Discussion

FAT10 is probably the most enigmatic ubiquitin-like protein, as little is known on its mechanism of activation, biological targets, and hence the physiological processes regulated by FAT10 conjugation. Using mass spectrometry, we identified two novel FATylation substrates, LULL1 and LRRFIP2 (Fig. 1). LULL1 is located mainly at the endoplasmic reticulum membrane and interacts with the

AAA + ATPase TorsinA to regulate its distribution and activity within the endoplasmic reticulum and nuclear envelope [19,20]. This activity is impaired in *DYT1* dystonia [20,21]. While the disease appears to be due to a mutation in TorsinA that may affect its association with LULL1, it will be interesting to test whether FATylation of LULL1 is necessary for this association. The second identified protein, LRRFIP2, attracted our attention because it is implied in the transduction of inflammation signals.

FATylation of LRRFIP2 requires UBA6, the specific FAT10 E1 [15,22], and occurs via monoFATylation at two distinct sites (Fig. 1). Interestingly, the first modification appears to enable the second one, and this interdependency seems to be attributed to a sensing mechanism embedded in FAT10: even a short N-terminal truncation of FAT10 abolishes the second modification of LRRFIP2 (Fig. 1E). LRRFIP2, LULL1 (data not shown) and p62 [15] appear to have a similar FATylation pattern, suggesting that modification by a few single FAT10 moieties is sufficient to alter the fate/function of the target protein. One of the target lysines on LRRFIP2 was identified as Lys276 (Table 1), though the site appears to be promiscuous, as substitution of this residue with arginine did not affect the FATylation pattern (not shown).

After stimulation of the TLR4 by LPS, LRRFIP2 is recruited to the receptor along with MYD88 to positively regulate NF-κB activation [17]. FATylation seems to interfere in this process by translocating the active phopshorylated form of LRRFIP2 from the membrane to the insoluble fraction of the cells (Fig. 2A). This is supported by the apparent co-localization of WT FAT10, but not of its FATylation-defective mutant, with LRRFIP2 in cytoplasmic speckles (Fig. 3). At that point it was important to show whether FAT10 modification, by causing LRRFIP2 translocation to the insoluble fraction, inhibits also the TLR4 signaling pathway. Indeed, we show that NF-κB activation mediated by LPS is inhibited significantly only when WT FAT10 is expressed (Fig. 4). This inhibition could be due to the insolubilization and therefore inactivation of LRRFIP2. The fate of the sequestered LRRFIP2 remains unclear. Given that no deFATylating enzymes have been described [9], the efficient proteasomal degradation of FAT10 [8] would be the only way to counteract its anti-inflammatory role, and to allow timely regulation of activation of NF-κB.

FAT10 is known to be induced under different conditions and in different cells by pro-inflammatory cytokines like IFNγ and TNFα [6,7]. Here we described an anti-inflammatory effect of FAT10. Thus it appears that the involvement of this ubiquitin-like protein in the inflammatory response is rather complex and multi-faceted. For example, it is possible that the cytokines induce the terminator of the inflammatory response along with the inflammatory process to ascertain its proper regulation. Alternatively, FAT10 may serve various roles at different stages of inflammation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.09.110.

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