ORIGINAL ARTICLE

Tissue Transglutaminase-Mediated AT$_1$ Receptor Sensitization Underlies Pro-inflammatory Cytokine LIGHT-Induced Hypertension

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BACKGROUND
Although numerous recent studies have shown a strong link between inflammation and hypertension, the underlying mechanisms by which inflammatory cytokines induce hypertension remain to be fully elucidated. Hypertensive disorders are also associated with elevated pressor sensitivity. Tissue transglutaminase (TG2), a potent cross-linking enzyme, is known to be transcriptionally activated by inflammatory cytokines and stabilize angiotensin II (Ang II) receptor AT$_1$ (AT$_1$R) *via* ubiquitination-preventing posttranslational modification. Here we sought to investigate the TG2-mediated AT$_1$R stabilization in inflammation-induced hypertension and its functional consequences with a focus on receptor abundance and Ang II responsiveness.

METHODS AND RESULTS
Using an experimental model of inflammation-induced hypertension established by introducing the pro-inflammatory tumor necrosis factor cytokine LIGHT, we provide pharmacologic and genetic evidence that TG2 is required for LIGHT-induced hypertension (systolic pressure on day 6: LIGHT = 152.3 ± 7.4 vs. LIGHT+ERW1041E [TG2 inhibitor] = 105.8 ± 13.1 or LIGHT+TG2$^{-/-}$ = 114.3 ± 4.3 mm Hg, $P < 0.05$, $n = 4$–5) and renal compromise (urine albumin/creatinine: LIGHT = 0.17 ± 0.05 vs. LIGHT+ERW1041E = 0.03 ± 0.01 or LIGHT+TG2$^{-/-}$ = 0.06 ± 0.01 mg/mg; plasma creatinine: LIGHT = 1.11 ± 0.04 vs. LIGHT+ERW1041E = 0.94 ± 0.04 or LIGHT+TG2$^{-/-}$ = 0.88 ± 0.09 mg/dl; urine volume: LIGHT = 0.23 ± 0.1 vs. LIGHT+ERW1041E = 0.84 ± 0.13 or LIGHT+TG2$^{-/-}$ = 1.02 ± 0.09 ml/24 hour on day 14, $P < 0.05$, $n = 4$–5). Our mechanistic studies showed that the TG2-mediated AT$_1$R modification and accumulation (relative renal AT$_1$R level: phosphate-buffered saline [PBS] = 1.23 ± 0.22, LIGHT = 3.49 ± 0.37, and LIGHT+ERW1041E = 1.77 ± 0.46, $P < 0.05$, $n = 3$; LIGHT+TG2$^{+/+}$ = 85.28 ± 36.11 vs. LIGHT+TG2$^{-/-}$ = 7.01 ± 5.68, $P < 0.05$, $n = 3$) induced by LIGHT is associated with abrogated β-arrestin binding (AT$_1$R/associated β-arrestin ratio: PBS = 2.62 ± 1.07, LIGHT = 38.60 ± 13.91, and LIGHT+ERW1041E = 6.97 ± 2.91, $P < 0.05$, $n = 3$; LIGHT+TG2$^{+/+}$ = 66.43 ± 44.81 vs. LIGHT+TG2$^{-/-}$ = 2.45 ± 1.78, $P < 0.01$, $n = 3$) and could be found in renal medulla tubules of kidneys (relative tubular AT$_1$R level: PBS = 5.91 ± 2.93, LIGHT = 92.82 ± 19.54, LIGHT+ERW1041E = 28.49 ± 11.65, and LIGHT+TG2$^{-/-}$ = 0.14 ± 0.10, $P < 0.01$, $n = 5$) and the blood vasculature (relative vascular AT$_1$R level: PBS = 0.70 ± 0.30, LIGHT = 13.75 ± 2.49, and LIGHT+ERW1041E = 3.28 ± 0.87, $P < 0.01$, $n = 3$), 2 of the tissues highly related to the genesis of hypertension. Our *in vitro* cellular assays showed that LIGHT stimulation triggered a rapid TG2-dependent increase in the abundance of AT$_1$Rs (relative AT$_1$R level after 2-hour LIGHT treatment: AT$_1$R (WT)+TG2 = 2.21 ± 0.23, AT$_1$R (Q315A)+TG2 = 0.18 ± 0.23, $P < 0.05$ vs. starting point = 1, $n = 2$) and downstream calcium signaling (fold increase in NFAT-driven luciferase activity: Saline = 0.02 ± 0.03, Ang II = 0.17 ± 0.08, LIGHT = 0.05 ± 0.04, LIGHT+Ang II = 0.90 ± 0.04 ($P < 0.01$ vs. Ang II), and LIGHT+Ang II+ERW1041E = 0.15 ± 0.15 ($P < 0.01$ vs. LIGHT+Ang II), $n = 3$).

CONCLUSIONS
Our data indicate an essential and systemic role for TG2 in bridging inflammation to hypertension *via* its posttranslational modifications stabilizing AT$_1$ receptor and sensitizing Ang II. Our findings also suggest that TG2 inhibitors could be used as a novel group of cardiovascular agents.

Keywords: ACE–angiotensin receptors–renin angiotensin system; blood pressure; GPCR; hypertension; inflammation; tissue transglutaminase.

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Hypertension is a major risk factor for cardiovascular disorders in millions of individuals worldwide.[1–3] Research has traditionally focused on the renin–angiotensin–aldosterone system, renal function, endothelial cells, sympathetic nervous system, and genetics[4] to understand the multitude of factors contributing to hypertension. More recently, the contribution of pro-inflammatory cytokines that are significantly elevated in multiple hypertensive conditions has been recognized. These cytokines include interleukin-1β,[5] tumor necrosis factors,[6,7] interleukin-6,[8] and interleukin-17.[9] A causal role for these cytokines in hypertension is supported by experiments with rodents showing that introduction of these cytokines results in increased blood pressure.[7,10–14] The essential and intrinsic role of inflammation in hypertension has been summarized and highlighted in several recent reviews.[15–19] Despite considerable evidence that inflammatory cytokines cause hypertension, the molecular mechanisms underlying cytokine-induced hypertension remain to be fully elucidated.

To understand how cytokines cause hypertension, we developed an experimental model of hypertension established by the introduction of the pro-inflammatory cytokine LIGHT, a member of the tumor necrosis factor superfamily (TNFSF14).[7] LIGHT triggers inflammatory responses by activating 2 widely distributed receptors, the herpes virus entry mediator[20] and the lymphotoxin β receptor,[21,22] upstream of the NFκB signaling pathway.[23,24] Using this experimental model of inflammation-induced hypertension, we discovered that transglutaminase is a critical link in this process.[25] Transglutaminases are a family of cross-linking enzymes that catalyze the formation of inter- or intramolecular ε-(γ-glutamyl)-lysine isopeptide bonds or the incorporation of primary amines on peptide-bound glutamine residues in a calcium-dependent manner.[26–29] Among the transglutaminase enzyme family, tissue transglutaminase (TG2) is the most ubiquitous member and present in most cell types.[28] The potent enzyme is involved in multiple cardiovascular disorders including atherosclerosis,[30–33] cardiac hypertrophy,[34,35] and chronic kidney disease/renal fibrosis[36–38] by cross-linking extracellular matrix proteins.[39–42] Our previous study also found TG2 plays an essential role in an autoantibody-induced model of preeclampsia (pregnancy-induced hypertension) in mice and stabilizes angiotensin II (Ang II) receptor AT_1 (AT_1R) *via* ubiquitination-preventing posttranslational isopeptide modification.[43] Of note, β-arrestin, the key adaptor protein in the desensitization of G-protein-coupled receptors (GPCRs), has long been shown to participate in the receptor ubiquitination process.[44] These facts drove us to hypothesize that in LIGHT-induced hypertension, the TG2-mediated posttranslational modification (PTM) of AT_1Rs may impair the receptor's desensitization process, finally contributing to increased Ang II sensitivity, a phenomenon consistently observed in multiple hypertensive patient groups and animal models.[45–49] In this study, we present evidence that TG2 is an essential and systemic contributor to the inflammatory cytokine LIGHT-induced hypertension *via* PTMs that stabilize AT_1 receptors and result in increased Ang II sensitivity.

METHODS

Please see Supplementary Data.

RESULTS

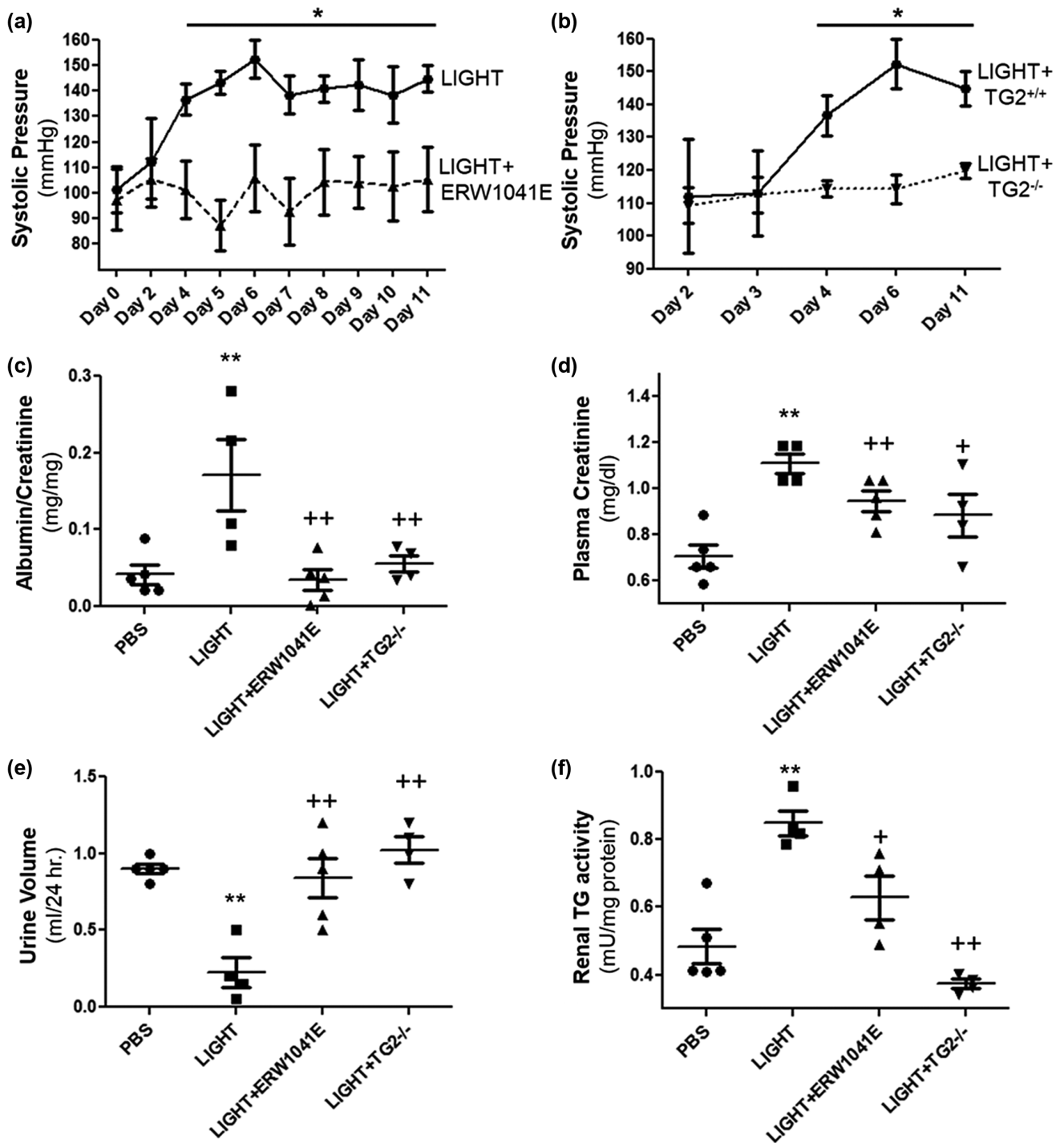### TG2 is required for LIGHT-induced hypertension and renal impairment

To specifically address the role of TG2 in LIGHT-induced hypertension, we inhibited its enzyme activity with the specific inhibitor ERW1041E or abrogated its expression with genetic ablation in the animal model. LIGHT-induced increase in blood pressure was significantly ameliorated in mice co-treated with ERW1041E, as measured by tail-cuff plethysmography (Figure 1a). Consistent with the finding from the Ang II model,[50] TG2-deficient mice are also resistant to the LIGHT-induced increase in blood pressure (Figure 1b). These results indicate an essential role for TG2 in LIGHT-induced hypertension.

Because renal impairment is associated with the progression of hypertensive disorders, we next looked into renal functions in the treated mice. LIGHT treatment resulted in severe albuminuria that was evident at the end of the 14-day treatment compared with the control mice treated with phosphate-buffered saline vehicle. In contrast, the ERW1041E treatment or TG2 ablation significantly attenuated the LIGHT-induced albuminuria (Figure 1c). In addition to proteinuria, a decline in renal function is usually associated with elevated plasma creatinine and reduced urine volume.[51] Consistent with the proteinuria data, the LIGHT-induced plasma creatinine accumulation and reduction in urine volume were significantly ameliorated in the ERW1041E-treated mice and TG2-deficient mice (Figure 1d and e). Altogether, these results indicate that TG2 is required for LIGHT-induced renal impairment.

To further address the involvement of renal TG2 in these processes, we then assessed the effect of TG2 inhibition or genetic ablation on LIGHT-induced increase in renal TGase activity. A significant repression in LIGHT-induced renal TGase activity was observed in ERW1041E-treated mice or TG2-deficient mice compared to controls treated with LIGHT only (Figure 1f) as measured by *in vitro* TGase assay, indicating TG2 as a major source or requirement for the LIGHT-induced increase in kidney TGase activation. Altogether, the pharmacologic and genetic data presented in Figure 1 suggest an essential role of TG2 in LIGHT-induced hypertension and renal compromise.

LIGHT induces TG2-dependent accumulation of AT1 receptor dissociated with β-arrestin in kidneys

To determine if the elevated TG2 in kidneys of LIGHT-treated mice[25] resulted in the TG2-mediated accumulation of AT_1Rs,[43] we measured the relative receptor abundance in the kidneys of LIGHT-treated mice with or without ERW1041E treatment or TG2 ablation. Western blot analysis showed that there was a significant increase in renal AT_1Rs in LIGHT-treated animals compared to phosphate-buffered saline controls (Figure 2a). Treatment with TG2 inhibitor

Figure 1. TG2 is required for LIGHT-induced hypertension and renal dysfunction. TG2 inhibitor ERW1041E treatment (**a**) or genetic ablation (**b**) significantly attenuated LIGHT-induced increase in blood pressure. Systolic blood pressure of mice injected with LIGHT (4 ng/day) in the presence or absence of the TG2 inhibitor ERW1041E (0.125 mg/day) or genetic ablation was determined on the days indicated by tail cuff plethysmography. (*$P < 0.05$ vs. LIGHT+ERW1041E or LIGHT+ TG2$^{-/-}$; $n = 4$ or 5 mice per group). TG2 inhibitor ERW1041E treatment or genetic ablation also significantly ameliorated LIGHT-induced proteinuria (**c**), plasma creatinine accumulation (**d**), urine retention (**e**), and renal TGase activation (**f**) (**$P < 0.01$ vs. PBS; $^{+}P < 0.05$, $^{++}P < 0.01$ vs. LIGHT). All mice in panels **c–f** were treated for 14 consecutive days prior to measurements ($n = 4$ or 5 mice per group). Abbreviations: PBS, phosphate-buffered saline; TG2, tissue transglutaminase.

ERW1041E or genetic ablation of TG2 abrogated the LIGHT-induced AT$_1$R accumulation in kidneys (Figure 2a and b). To determine if the elevated AT$_1$Rs were modified

by TG2 and resistant to β-arrestin-mediated desensitization, we immunoprecipitated AT$_1$Rs from the renal lysates of the LIGHT-treated mice (Figure 2a and b). From the pull-down
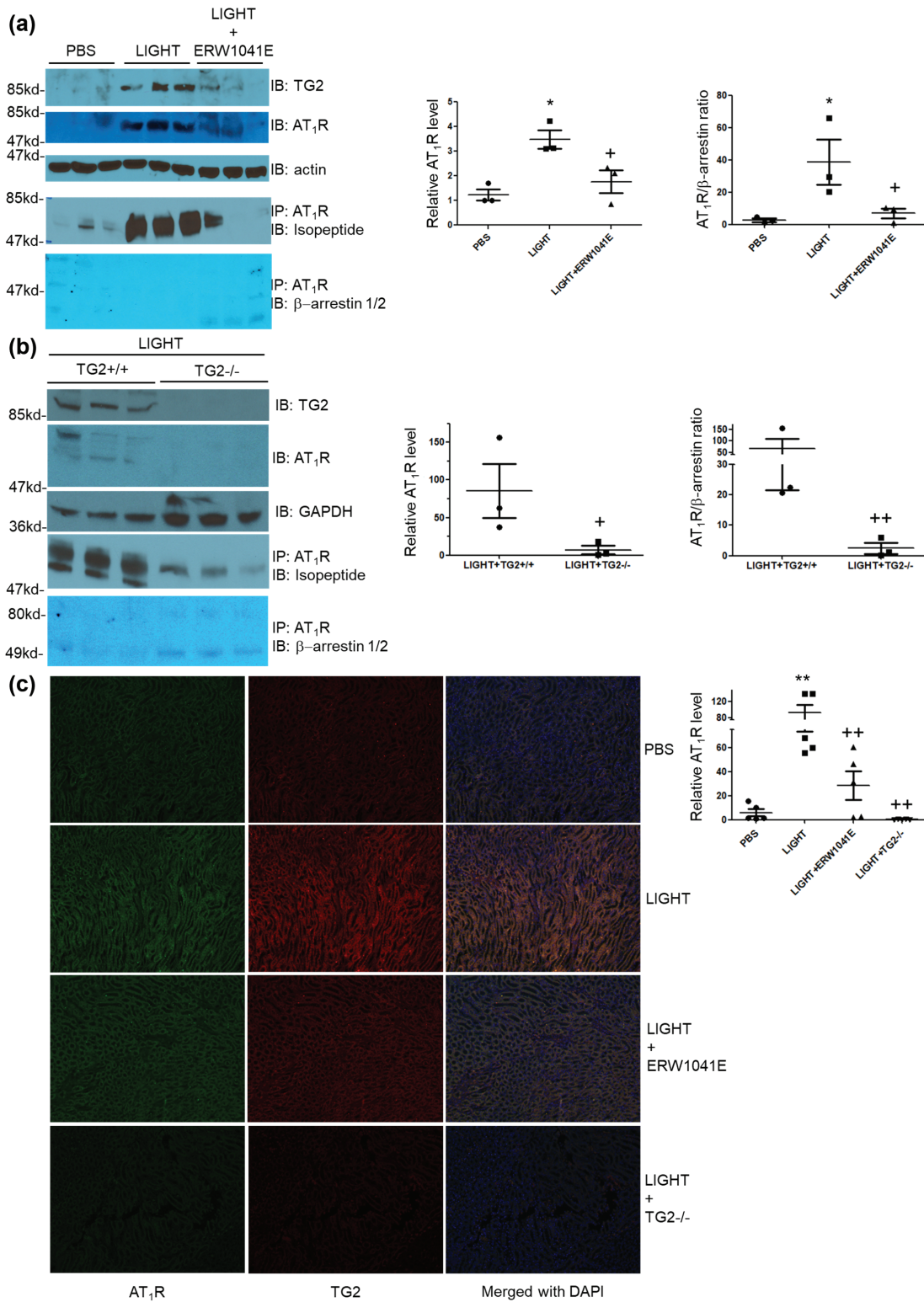
Figure 2. TG2-mediated LIGHT-induced AT$_1$ receptor accumulation and dissociation with β-arrestin in kidneys. (**a**) LIGHT treatment resulted in a significant increase in renal AT$_1$ receptors with TG2 modification, and dissociated from β-arrestin that was attenuated by TG2 inhibitor ERW1041E. (**b**) LIGHT-induced increase in AT$_1$ receptor abundance, isopeptide modification, and dissociation with β-arrestin was abrogated in TG2$^{-/-}$ mice. (**c**) Increased TG2 and AT$_1$Rs were co-localized in renal medulla tubules of LIGHT-treated animals, but not those co-treated with ERW1041E or with TG2 genetic ablation (n = 5 in each group). (*$P < 0.05$, **$P < 0.01$ vs. PBS; $^+P < 0.05$, $^{++}P < 0.01$ vs. LIGHT). Abbreviations: PBS, phosphate-buffered saline; TG2, tissue transglutaminase.

products, we found an increased level of AT_1Rs with TGase-mediated isopeptide modification and abrogated β-arrestin association in LIGHT-treated animals that was repressed by either ERW1041E treatment or TG2 ablation (Figure 2a and b), suggesting that TG2 played a crucial role in LIGHT-induced AT_1 receptor accumulation and sensitization in kidneys. Using immunofluorescent staining, we co-localized increased TG2 and AT_1Rs in the renal medulla tubules of LIGHT-treated animals but not in mice co-treated with ERW1041E or in mice with genetic ablation of TG2 (Figure 2c). Taken together, data presented in Figure 2 indicate that activation/elevation of TG2 in the renal medulla leads to accumulation of AT_1Rs with TG2-mediated isopeptide modification.

Besides renal dysfunction, vascular perturbation has long been recognized as another major contributor to the genesis of hypertension. Thus in this study, we also examined the TG2-mediated AT_1R accumulation in blood vessels using immunofluorescent staining. As shown in Supplementary

Figure 1, LIGHT treatment resulted in TG2-dependent AT_1R elevation in pulmonary vasculature. Interestingly, we also observed a TG2-dependent AT_1R accumulation in the smooth muscle cell layer of the bronchial airways of LIGHT-treated animals, indicating the event's ubiquitous presence in cells with AT_1R and TG2 expression.

LIGHT stimulation causes TG2-dependent AT_1 receptor accumulation and Ang II sensitization

To investigate the kinetics of the TG2-dependent AT_1R accumulation induced by LIGHT, we carried out studies with the human trophoblast cell line, *HTR*-8/SVneo, which is characterized by high expression levels of both AT_1Rs and TG2[43] mimicking their upregulation in the renal tubular or vascular cells exposed to inflammation and/or hypoxia. And these cells also express membrane receptors for LIGHT, the herpes virus entry mediator and the lymphotoxin β receptor.[7] Our results showed a dose-dependent LIGHT-induced
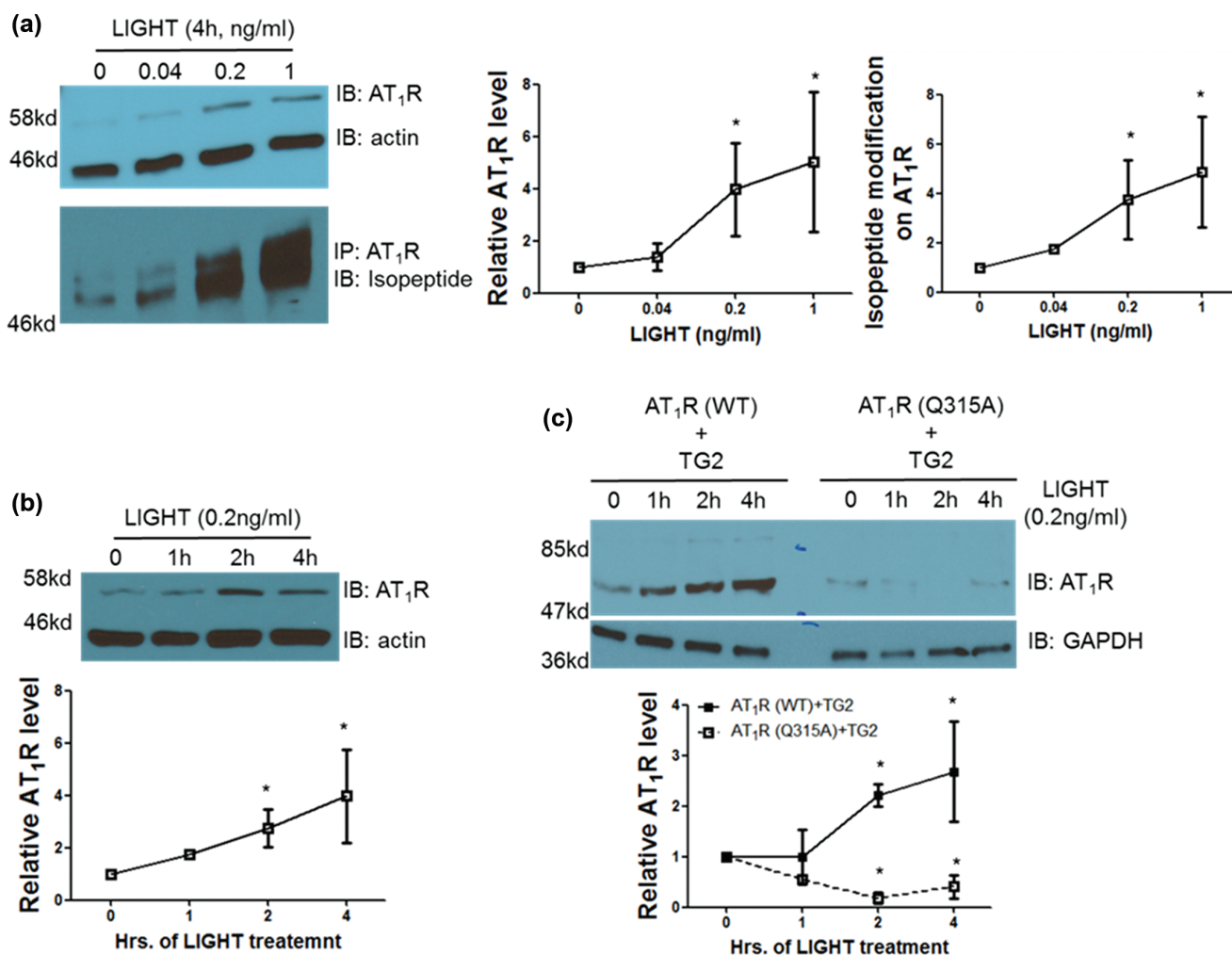
Figure 3. LIGHT stimulation stabilizes AT_1 receptor *via* TG2-mediated isopeptide modification at Q315. (**a**) LIGHT stimulation stabilized AT_1 receptor with ε-(γ-glutamyl)-lysine isopeptide modification in a dose-dependent fashion in HTR cells (*$P < 0.05$ vs. 0; $n = 2$–3). (**b**) LIGHT-induced AT_1 receptor stabilization peaked within 2-hour treatment in HTR cells (*$P < 0.05$ vs. 0; $n = 2$–3). (**c**) LIGHT-induced AT_1R accumulation was abolished in Chinese hamster ovary cells overexpressing TG2 and AT_1R Q315A mutant but not those overexpressing TG2 and wild-type AT_1R (*$P < 0.05$ vs. 0, $n = 2$). Abbreviations: PBS, phosphate-buffered saline; TG2, tissue transglutaminase.
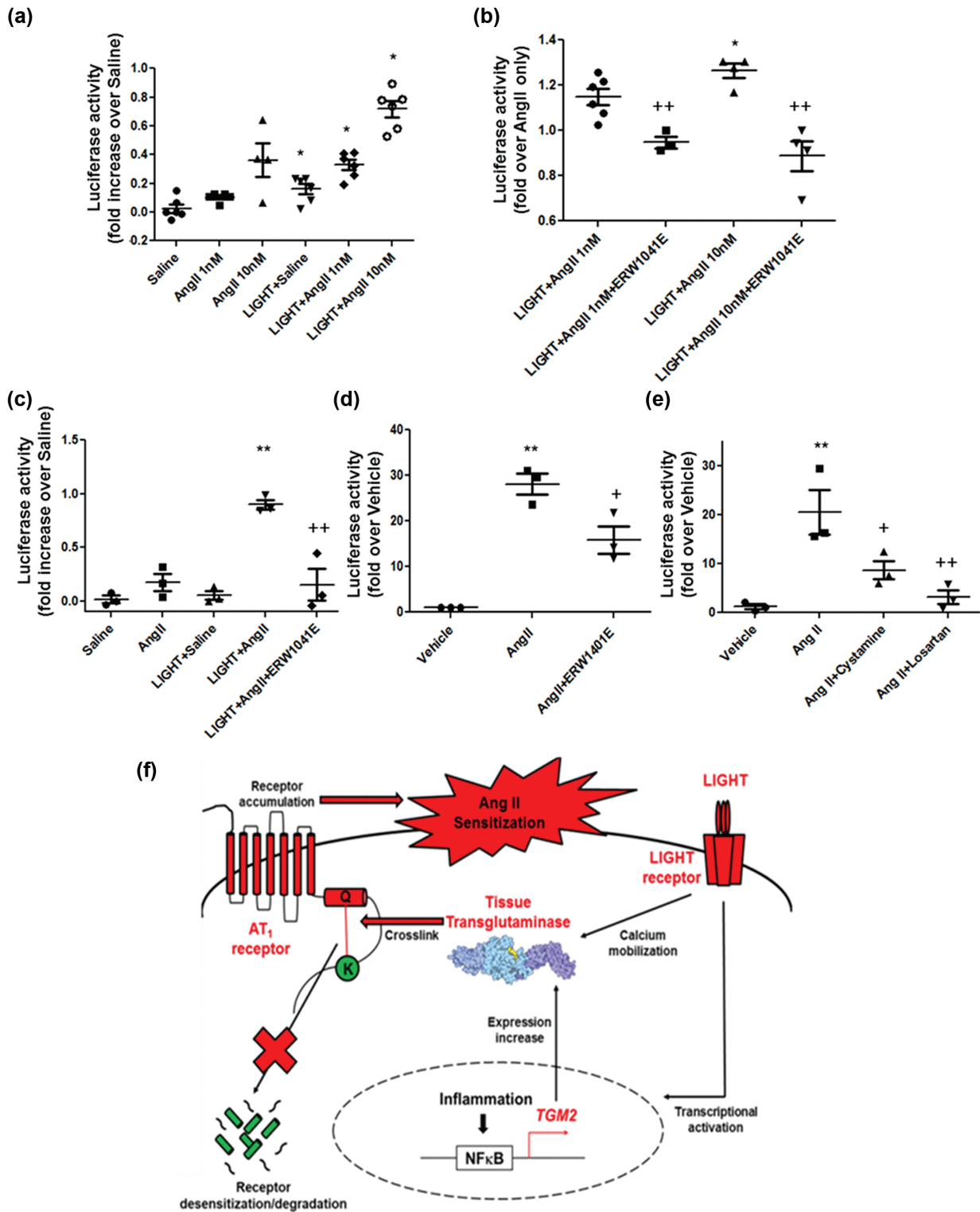
Figure 4. TG2 contributes to LIGHT-induced Ang II sensitization. (**a**) Ang II-induced calcium response was significantly pronounced in AT_1R-NFAT-luciferase reporter cells pretreated with LIGHT (200 pg/ml) for 2 hours ($n = 4–6$; *$P < 0.05$ vs. treatment w/o LIGHT pretreatment; duration of Ang II incubation is overnight at the indicated dose). (**b**) TG2 inhibitor ERW1041E inhibited the enhanced Ang II response triggered by 2-hour LIGHT (200 pg/ml) pretreatment ($n = 3–6$; *$P < 0.05$ vs. LIGHT+AngII 1nM; ++$P < 0.01$ vs. w/o ERW1041E (10 μM) co-pretreatment; duration of Ang II incubation is overnight at the indicated dose). (**c**) Low-dose Ang II (1 nM)-induced calcium response was enhanced by low-dose LIGHT (50 pg/ml) but not low-dose LIGHT (50 pg/ml) plus TG2 inhibitor ERW1041E (10 μM) co-incubation ($n = 3$, **$P < 0.01$ vs. Ang II; ++$P < 0.01$ vs. LIGHT+Ang II; duration of all the indicated treatments is overnight). TG2 inhibitor ERW1041E (200 μM) (**d**) or TGase inhibitor cystamine (500 μM) (**e**) significantly attenuated high-dose Ang II (100 nM)-induced

accumulation of AT_1Rs that started to peak at 0.2 ng/ml LIGHT after a 4-hour treatment and was accompanied with an increase in the level of ε-(γ-glutamyl)-lysine isopeptide modification of the receptor (Figure 3a). Using the peak dose of 0.2 ng/ml LIGHT, we next observed an increase in the abundance of AT_1Rs after 2 hours in a time-course treatment (Figure 3b). To determine if the LIGHT-induced accumulation of AT_1Rs depended on TG2 modification, we provided similar LIGHT stimulation to Chinese hamster ovary cells overexpressing TG2 and either wild-type or the Q315A mutant AT_1R in which the glutamine residue in the cytoplasmic tail of the receptor for TG2 modification is replaced with an alanine.[43,52] LIGHT stimulation resulted in a time-dependent accumulation (Figure 3c) of AT_1Rs in cells overexpressing wild-type receptors but not the Q315A mutant where exacerbated ubiquitination-dependent receptor degradation could be observed with the help of proteasome inhibitor MG132 (data not shown). Taken together, these results show that LIGHT stimulation causes a TG2-mediated increase in the abundance of AT_1Rs by interfering with their ubiquitination-dependent degradation.

To explore the functional consequences of LIGHT-induced TG2 modifications on AT_1R signaling, we assessed the Ang II-induced downstream calcium response by measuring luciferase activity in AT_1R-NFAT-luciferase reporter cells.[53] Initially, the reporter cells were incubated with or without LIGHT (200 pg/ml) for 2 hours followed by overnight incubation with Ang II (1 or 10 nM). The luciferase assay results (Figure 4a) indicate that preincubation with LIGHT resulted in a significantly higher calcium response following Ang II stimulation. TG2 inhibitor ERW1041E co-pretreatment was able to prevent the increase in the follow-up Ang II responsiveness (Figure 4a). Prolonged incubation with LIGHT at a lower dose (50 pg/ml, overnight) resulted in a more pronounced enhancement of the low-dose (1 nM) Ang II-induced luciferase expression (Figure 4b). Importantly, this LIGHT-induced hypersensitivity to Ang II was prevented by the TG2 inhibitor ERW1041E (Figure 4c), indicating the crucial role of LIGHT-induced TG2 modifications in this process. To further assess the endogenous role of TG2 in AT_1R signaling, we treated the reporter cells with a high-dose Ang II (100 nM) in the presence or absence of TG2 inhibitor ERW1041E or the pan-TGase inhibitor cystamine. The results (Figure 4d and e) show that Ang-II-induced calcium signaling was significantly inhibited by ERW1401E or cystamine, suggesting that TG2 may function as a downstream enhancer in the endogenous Ang II–AT_1R signaling pathway.

DISCUSSION

In this report, we provide pharmacologic and genetic evidence that TG2 is required for pro-inflammatory cytokine LIGHT-induced hypertension. Our mechanistic studies indicated that the TG2-mediated AT_1R modification and accumulation induced by LIGHT is associated with abrogated β-arrestin binding and could be found in renal medulla tubules of kidneys and the blood vasculature. *In vitro* cellular assays showed that the TG2-dependent accumulation of AT_1Rs contributes to Ang II sensitization. Taken together, our findings reveal a previously unrecognized role of the inflammatory signals in elevating pressor sensitivity *via* TG2-mediated PTMs. Our results may also suggest a systemic mechanism of TG2-mediated enhancement of AT_1R signaling in hypertensive disorders linked with inflammation.

Kidney is one of the central organs in the regulation of blood pressure, and its malfunction results in hypertension.[54–56] Our previous results indicate that renal TGase activation is associated with hypertension and renal impairment induced by LIGHT. We report here that LIGHT treatment caused upregulation of TG2 and increased modification of AT_1Rs in the renal medulla (Figure 2) where a hypoxic environment may be maintained or even pronounced.[57,58] The presumably hypoxic and inflammatory environment of renal medulla of LIGHT-treated animals stimulates the activation of TG2 that in turn resulted in AT_1R accumulation. Of note, over-activation of renal renin–angiotensin–aldosterone machinery is considered as a prominent contributing factor of essential hypertension,[59–61] and perturbation in medullary tubules has also been recognized as the driving force of salt and water retention. In this way, enhanced Ang II signaling caused by TG2-mediated AT_1R accumulation in medullary tubules may contribute to the downstream outputs favoring hypertension. Consistently, LIGHT-induced accumulation of TG2-modified AT_1Rs in kidneys was associated with impaired renal function as evident from increased urine retention, albuminuria, and elevated plasma creatinine, and the renal impairment was prevented in TG2-deficient mice or in mice treated with the TG2 inhibitor ERW1041E. Besides kidney, previous studies from us and others[25,50,62] suggest the possible involvement of vascular TG2 in hypertension, and the TG2-dependent AT_1R accumulation was also found in endothelial and smooth muscle cells of the vasculature (Supplementary Figure 1). Given the ubiquitous presence of Ang II–AT_1R signaling axis among related organ/tissues including kidney, vasculature,[63] T lymphocytes,[64] and central nervous system,[65] our findings may suggest a TG2-mediated general mechanism downstream of inflammation for the genesis of hypertension. Mice with cell-type-specific deletions will be especially important to determine the cellular source of TG2 that is required for LIGHT-induced pathophysiology.

Elevated pressor sensitivity is consistently observed among multiple hypertensive patient groups and animal models,[45–49] and the contributing role of inflammatory

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calcium signaling in AT_1R-NFAT-luciferase reporter cells ($n = 3$, **$P < 0.01$ vs. Vehicle; +$P < 0.05$, ++$P < 0.01$ vs. Ang II; duration of all the indicated treatments is overnight; losartan = 2 μM). (**f**) Mechanistic model for a role of TG2 in LIGHT-induced cardio-renal syndrome: By activating its membrane receptors, the TNF cytokine LIGHT transcriptionally increases *TGM2* gene expression and/or directly activates TG2 *via* calcium mobilization. TG2-mediated modification of AT_1Rs at Q315 impairs receptor desensitization/proteasomal degradation, contributing to Ang II sensitization. Abbreviations: Ang II, angiotensin II; TG2, tissue transglutaminase; TNF, tumor necrosis factor.

cytokines in this process has been noticed.[66,67] Also, people have long been intrigued by the accumulation of pressor receptors including alpha-adrenergic receptor[68–71] and AT_1R[72] in hypertensive animals. Together with the studies[50] addressing the role of TG2 in vascular remodeling and endothelial function, our studies might provide a mechanistic insight into the intrinsic link among these phenomena. We found that the inflammatory cytokine LIGHT treatment leads to a TG2-dependent elevation of AT_1R with abrogated β-arrestin association in kidneys. To explore its functional consequences, we used overexpression cell lines to mimic the cell types with high levels of AT_1R and TG2. With them, we showed that the rapid TG2-dependent accumulation of AT_1Rs induced by LIGHT is associated with elevation of Ang II sensitivity. As a 7 transmembrane receptor, AT_1R is regulated by GPCR kinases (GRKs) and β-arrestin-mediated desensitization.[73] The increased ligand sensitivity and abrogated arrestin association suggest the inhibitory effect of TG2-mediated receptor modification on the desensitization process. The TG2-dependent AT_1R accumulation further supports this concept if β-arrestin-mediated receptor ubiquitination could be considered as a long-term outcome of the desensitization.[44] Consistently, transglutaminase inhibitors dampened Ang II-induced signaling response. Taken together, these results are suggesting an intrinsic role of TG2 in the maintenance of endogenous GPCR signaling.

We have recently shown that LIGHT-induced TG2 triggers the production of AT_1R activating autoantibodies (AT_1-AA) that contribute to hypertension and renal impairment. These autoantibodies were originally observed in women with preeclampsia and subsequently observed in other hypertensive conditions. These pathogenic autoantibodies (in humans and mice) recognize a common epitope, AFHYES**Q**, present on the second extracellular loop of the AT_1R. Thus, one possible mechanism by which LIGHT-induced TG2 contributes to hypertension is by PTM of the epitope glutamine (**Q187**) thereby creating a neoantigen that contributes to AT_1-AA production *via* the adaptive immune response. However, this process requires activation of the adaptive immune system, and is expected to take longer time. Another, more rapid mechanism by which TG2 may contribute to LIGHT-induced hypertension is by PTM of AT_1Rs at Q315. Modification of this glutamine by TG2 prevents ubiquitin-dependent proteasomal degradation, thereby allowing AT_1Rs to accumulate quickly (~2 hours). Modification at this site also blocks β-arrestin binding, resulting in prolonged receptor activation. Increased AT_1R abundance and reduced β-arrestin binding (due to TG2-mediated PTM at Q315) occur more rapidly than the extended time required to activate the adaptive immune system to produce AT_1-AA (due to TG2-mediated PTM at Q187). It is also noteworthy that the TG2-mediated receptor modification at Q315 may enhance the autoimmune response because of increased receptor abundance. Moreover, our recent studies[43] also suggest that AT_1-AA is able to generate biased downstream signaling favoring receptor accumulation. Taken together, our data suggest a pathogenic vicious cycle is formed between the receptor accumulation and autoantibody generation under inflammatory conditions.

DISCLOSURE

The authors declared no conflict of interest.

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