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# Agonist-induced activation of human FFA1 receptor signals to extracellular signal-regulated kinase 1 and 2 through Gq- and Gi-coupled signaling cascades

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## Abstract

**Background:** FFA1 is abundantly expressed in the liver, skeletal muscle, monocytes and nervous system, but is particularly abundant in pancreatic  $\beta$  cells. It is widely believed that FFA1 exerts its regulatory roles in a variety of physiological and pathological functions. In response to oleic acid, FFA1 has been shown to induce the activation of extracellular signal-regulated kinase 1 and 2 (ERK1/2) through a mechanism involving EGFR transactivation in a breast cancer cell line. However, the underlying molecular mechanism for ERK1/2 activation mediated by n-6 free fatty acid (LA) in HEK293 cells remains to be further elucidated.

**Methods:** A FLAG-FFA1 vector was stably expressed in HEK293 cells. Western blot analysis was applied to investigate the change in LA-induced ERK1/2 phosphorylation change in response to kinase inhibitors. Arrestin-2/3-specific siRNA was used to analyze the effect of arrestin-2/3 knockdown on FFA1-mediated ERK1/2 activation.

**Results:** We proved that activation of ERK1/2 by LA was rapid, peaking at 5 min. Further experiments proved that FFA1 couples to a Gq protein and activates PI-PLC, which induces the IP3/Ca<sup>2+</sup> and DAG/PKC signal pathways, both of which are involved in ERK1/2 activation. We also showed that there is no EGFR transactivation, arrestin-2/3 or G $\beta$  $\gamma$  pathway participation in ERK1/2 phosphorylation. Treating cells with PTX abolished ERK1/2 activation at a late time point ( $\geq 20$  min), indicating a critical role for Gi subunits in FFA1-mediated ERK1/2 activation.

**Conclusions:** Our study provides a detailed delineation of the LA-mediated activation of ERK1/2 in HEK293 cells that are stably transfected with human FFA1. We also present evidence of Gi/Gq-induced synergism in the regulation of ERK1/2 phosphorylation. These observations may provide new insights into the pharmacological effects of FFA1 and the physiological functions modulated by FFA1-mediated activation of ERK1/2.

**Keywords:** FFA1, Phosphatidylinositol-specific phospholipase C, Extracellular signal-regulated kinase 1 and 2, Gq $\alpha$ /11, Gai/o

## Background

FFA1 was sequenced and identified as a member of a subfamily of intronless GPCRs. The family includes GPR41, GPR42 and GPR43, all of which reside on chromosome



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19q13.1. The receptor encoded by FFA1 contains two N glycosylation sites (N-X-S/T), five protein kinase C (PKC) phosphorylation sites, and a cysteine at the C-terminus [1]. Both saturated and unsaturated medium- and long-chain (C12-C22) free fatty acids (FFA) were identified as endogenous ligands for FFA1 [2]. FFA1 is abundantly expressed in rodent insulin-secreting cell lines, including INS-1E cells [3], Min6 cells [4] and pancreatic  $\beta$  cells [2]. It is also found in the human brain [5]. Accumulating evidence shows that FFA1 plays a crucial role in the regulation of glucose homeostasis mediated by free fatty acid-induced insulin secretion [6, 7]. In addition to the regulation of endocrine function, FFA1 is involved in bone remodeling, inflammation and neurogenesis [8–10]. These findings suggest that FFA1 may work through multiple pathways in the regulation of different physiological functions.

The basic understanding of G-protein-coupled receptors (GPCRs) is that almost all signal through extracellular signal-regulated kinase (ERK) signaling cascades, which are associated with about 200 cellular substrates and mediate a variety of cellular processes, including proliferation, differentiation, migration, survival and apoptosis [11–13].

FFA1 is activated to elicit an increase in intracellular  $\text{Ca}^{2+}$  levels via the Gq-dependent pathway, leading to enhancement of glucose-stimulated insulin secretion [14, 15]. In addition, FFA1 has been shown to induce ERK1/2 activation through a mechanism involving Src kinase and EGFR transactivation by oleic acid in the breast cancer cell lines MCF-7 and MDA-MB-231 [16]. By contrast, another study revealed that FFA1 activation by n-3 fatty acids can abolish EGF-induced proliferation and migration in MCF-7 and MDA-MB-231 cells [17]. This suggests that the effects of different FFA1 agonists are different.

In mouse embryonic stem cells, LA regulates various cell cycle proteins via p44/42 MAPK signaling [18]. Interestingly, unsaturated fatty acids promote the activation of ERK1/2 mainly via FFA1, leading to an anti-lipoapoptotic effect on NIT-1 cells [19]. Thus, the underlying mechanism regulating ERK1/2 activation mediated by the n-6 free fatty acids (LA) in HEK293 cells through FFA1 remains unclear.

It is now known that GPCRs regulate MAPK cascades via distinct  $\text{Gi}$ -,  $\text{Gs}$ -,  $\text{Gq/11}$ - and  $\text{G}\beta\gamma$ - dependent signaling pathways, leading to activation of ERK1/2 [20]. G proteins have complex and diverse roles in the FFA1 signal pathway. Pharmacological inhibition of  $\text{Gq/11}$  blocked  $\text{Ca}^{2+}$  release from the ER in the  $\beta$  cell line INS-1E [3]. In HEK cells, TUG424-mediated FFA1 downstream signaling is inhibited by pertussis toxin (PTX), which indicates that  $\text{Gi/o}$  is partially involved [21]. Moreover, LA have been proven to decrease the voltage-gated  $\text{K}^+$  current through FFA1/ $\text{Gs}$ /cAMP/protein kinase A (PKA) [22]. Further exploration of G protein subunits in FFA1-mediated ERK1/2 activation will be important to better understand the role of FFA1 in various physiological functions.

## Methods

### Materials

Lipofectamine 2000, G418 and Opti-MEM were purchased from Invitrogen. Cell culture media and fetal bovine serum were obtained from Hyclone. Pertussis toxin (PTX), Go6983 and thapsigargin were purchased from Sigma. UBO-QIC was purchased from Dr. E. Kostenis at the University of Bonn. U0126, tyrphostin AG1478, GM6001, PP2 and ET-18-och3 were from Calbiochem. Monoclonal anti-FLAG antibody was

purchased from BD Biosciences Pharmingen. Anti-phospho-ERK1/2 (Thr-202/Tyr-204) and ERK1/2 antibodies and horseradish peroxidase-conjugated anti-rabbit IgG were from Cell Signaling Technology. Anti-tubulin antibody was from Beyotime. The  $\beta$ -adrenergic receptor kinase COOH domain (495-689aa), adrenergic and the  $G\alpha$  subunit of transducin plasmid were from the lab of Dr. Naiming Zhou in Zhejiang University.

#### Cell culture

FFA1 (GenBank accession no.NM\_005303.2) was cloned via PCR using human genomic DNA as a template, with the primers:

5'-AAGCTTGCCACCATGGACCTGCCCCCGCAGCTCTCC-3' (forward) and

5'-GGTACCGTCTTCTGGGACTTGCCCCCTTGCGT-3' (reverse).

The PCR products were inserted into the *HindIII* and *Bgl* sites of the pCMV-Flag vector. HEK293 cells stably expressing human FFA1 were grown in DMEM supplemented with 10% (v/v) fetal bovine serum and 800 mg/l G418. Plasmid constructs were transfected into HEK293 cells using Lipofectamine 2000 according to the manufacturer's instructions. All cells were incubated at 37 °C in a humidified atmosphere of 5% CO<sub>2</sub>, 95% air.

#### Small interfering RNAs and siRNA Transfection

Small interfering RNAs (siRNAs) for arrestin-2 and arrestin-3 were purchased as a SMART pool from Dharmacon RNA Technologies. The clathrin HC siRNA was from Santa Cruz Biotechnology. A nonspecific RNA was used as the control for all siRNA experiments. The arrestin and CHC siRNAs were transfected according to the manufacturer's instructions. Briefly, the first siRNA transfection was performed using Lipofectamine 2000 and Opti-MEM from Invitrogen. After 6–8 h, the cells were divided between new 6-cm dishes. On day 2, a second siRNA transfection was performed. After 24 h, transfected cells were divided for use in various assays.

#### Western blot analysis

Cells were plated on 6-well plates, grown to 80% confluence, rinsed with serum-free DMEM, and incubated overnight in serum-free medium. For PTX treatment, the cells were pretreated with 100 ng/ml PTX overnight prior to the ERK1/2 assay. Cells were preincubated with various inhibitors for 1 h before activation with the indicated ligands. Ligand incubation was ended by washing the cells with 2 ml of ice-cold PBS followed by the addition of the lysis buffer (1% Triton X-100, 1% deoxycholate and 0.1% SDS) at 4 °C on a rocker for 30 min. The lysates were centrifuged at 4 °C at 10000 g for 15 min. About 40  $\mu$ g protein underwent electrophoresis on a 10% SDS polyacrylamide gel, then was transferred to a PVDF membrane and immunoblotted using monoclonal anti-pERK1/2 antibody from Cell Signaling Technology. Blots were probed with horseradish peroxidase-labeled secondary antibodies, and chemiluminescence was produced using HRP substrate (Cell Signaling Technology). The working HRP substrate was prepared by combining equal volumes of luminol reagent and peroxide solution. The HRP substrate produces a high intensity signal with low background for detection of both high and low abundance proteins. The blots were stripped and reprobbed using an anti-total ERK1/2 (1:2000) monoclonal antibody as a control for protein loading.

To analyze the knockdown of siRNA-targeted proteins, siRNA-transfected Flag-FFA1-HEK293 cells were seeded in a 6-well plate and treated as described above. After blocking, the PVDF membranes were incubated with anti-arrestins (1/1000) and probed with horseradish peroxidase-labeled secondary antibodies. Chemiluminescence was detected using an HRP substrate (Cell Signaling Technology). The blots were stripped and reprobed using an anti-total ERK1/2 (1:2000) monoclonal antibody as a control for protein loading, and all the immunoblots were visualized and quantified using the Bio-Rad Quantity One imaging system.

#### Cell viability assay

Cell viability was evaluated using CCK8 assay reagent (Beyotime Biotechnology). The FFA1-HEK293 cells were plated on 96-well plates. After pretreatment with PTX (100 ng/ml) for the indicated time, the viability of the FFA1-HEK293 cells was assessed using CCK8 to detect the soluble orange formazan generated by mitochondrial dehydrogenases, according to the manufacturer's instructions. The colorimetric signal of each sample was detected and analyzed using a FLUOstar OPTIMA microplate reader (BMG LABTECH Inc.).

#### Data analysis

All results are expressed as means  $\pm$  S.E. of N. Statistical significance was determined using Student's t-test. Probability values less than or equal to 0.05 were considered significant.

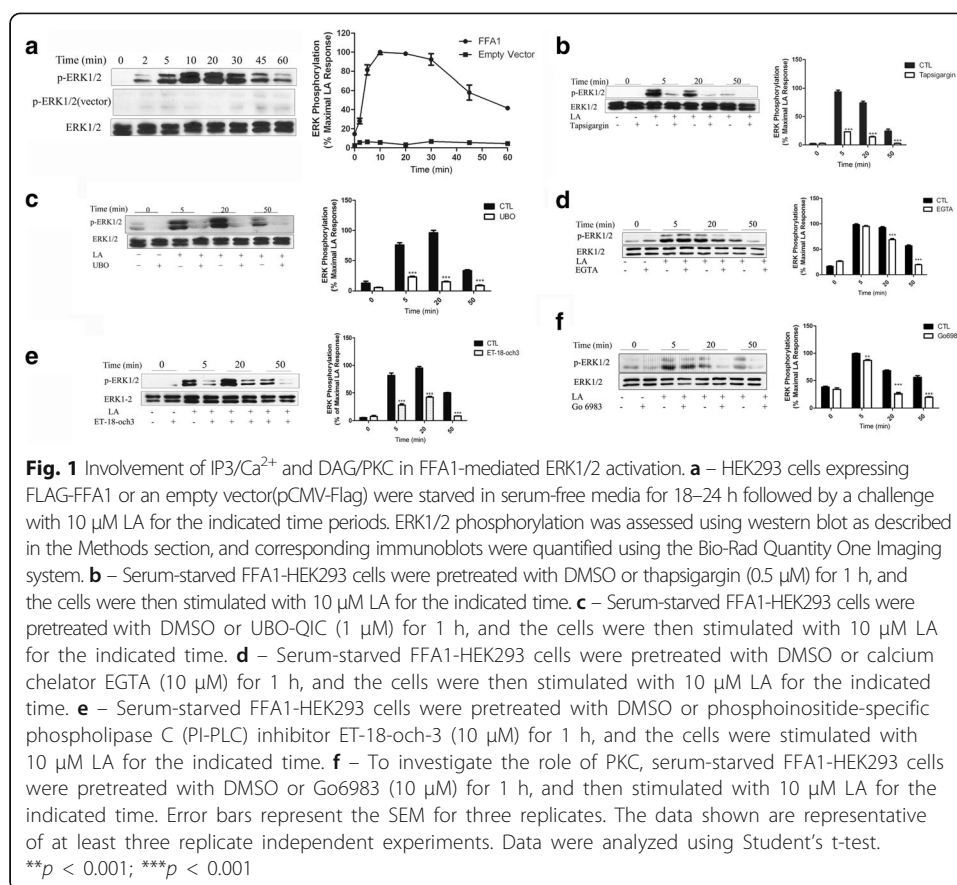
## Results

### Involvement of Gq/PI-PLC downstream signaling in ERK1/2 Phosphorylation

LA is known to be an endogenous ligand for FFA1. An expression vector containing human FFA1 fused with Flag-tag was constructed and stably expressed in HEK293 cells. Our recent studies demonstrated that the LA-induced internalization of FFA1 is regulated by G-protein-coupled receptor kinase 2 and arrestin-3. In addition, LA induced a concentration-dependent activation of ERK1/2 with an  $EC_{50}$  of 8.669  $\mu$ M in the stably transfected cells [23]. As illustrated in Fig. 1a, FFA1-initiated activation of ERK1/2 occurred in a time-dependent manner with a maximal activation at 10 min and with a subsequent reduction to base line by 60 min after stimulation with LA. Fasiglifam (TAK-875) enhances the glucose-stimulated insulin secretion (GSIS) pathway via IP<sub>3</sub>-induced Ca<sup>2+</sup> oscillations and the DAG/protein kinase C-independent (PKC-independent) Ca<sup>2+</sup> oscillations mechanism [24]. We speculated that Gq plays a crucial role in the process of FFA1 downstream ERK1/2 activation.

To investigate the role of Gq protein in FFA1-mediated activation of ERK1/2, cells were cultured in the presence or absence of UBO-QIC in DMEM. As shown in Fig. 1c, pretreatment with UBO-QIC resulted in nearly complete inhibition of ERK1/2. Pretreatment of FFA1-HEK293 cells with the PKC kinase inhibitor Go6983 for the time course shown in Fig. 1f, resulted in a significantly decrease in ERK1/2 at early ( $\leq$  5 min) and late time points ( $\geq$  5 min).

To assess whether IP<sub>3</sub>/Ca<sup>2+</sup> mobilization was involved in the LA-induced ERK1/2 signal pathway, cells were pre-incubated with the calcium chelator EGTA and endoplasmic reticulum Ca<sup>2+</sup> ATPase inhibitor thapsigargin. LA-stimulated ERK1/2



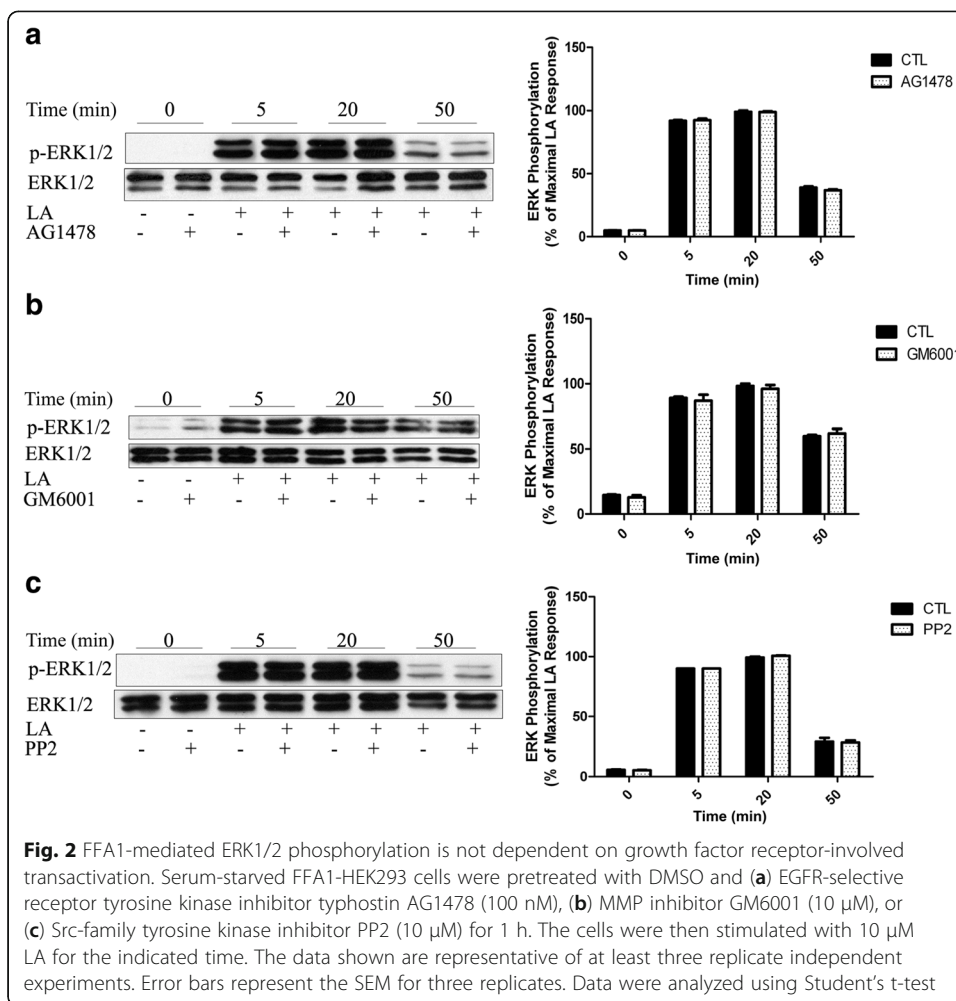
phosphorylation was significantly inhibited by EGTA and thapsigargin at late time points ( $\geq 20$  min; Fig. 1b and d).

To confirm that IP<sub>3</sub>-mediated calcium mobilization increases ERK1/2 phosphorylation, FFA1-HEK293 cells were pretreated with the phosphatidylinositol phospholipase C (PI-PLC) inhibitor edelfosine (ET-18-och3) for 1 h, followed by stimulation with LA for different lengths of time. Upon stimulation with LA, a significant inhibition of FFA1-mediated ERK1/2 activation was observed (Fig. 1e). Collectively, these data demonstrate that FFA1 mediates ERK1/2 signaling phosphorylation via dual mechanisms in which Gq/PI-PLC activates IP<sub>3</sub>/Ca<sup>2+</sup> and the DAG/PKC signaling pathway.

#### FFA1-mediated ERK1/2 Phosphorylation is not dependent on a Transactivation mechanism involving growth factor receptor

It is generally accepted that the transactivation of growth factor receptors participates in GPCR-mediated ERK1/2 phosphorylation [25]. Breast cancer cells are known to transactivate ERK1/2 through FFA1, involving Src and EGFR [16]. To assess the role of EGFR in the FFA1-induced ERK1/2 pathway in HEK293 cells, serum-starved FFA1-HEK293 cells were treated with AG1478 (EGF receptor inhibitor) and GM6001 (MMP inhibitor), followed by stimulation with LA.

As shown in Figs. 2a and b, inhibition of EGFR and MMP had no effect on ERK1/2 activation in response to LA in HEK293 cells stably expressing FFA1. Similar results

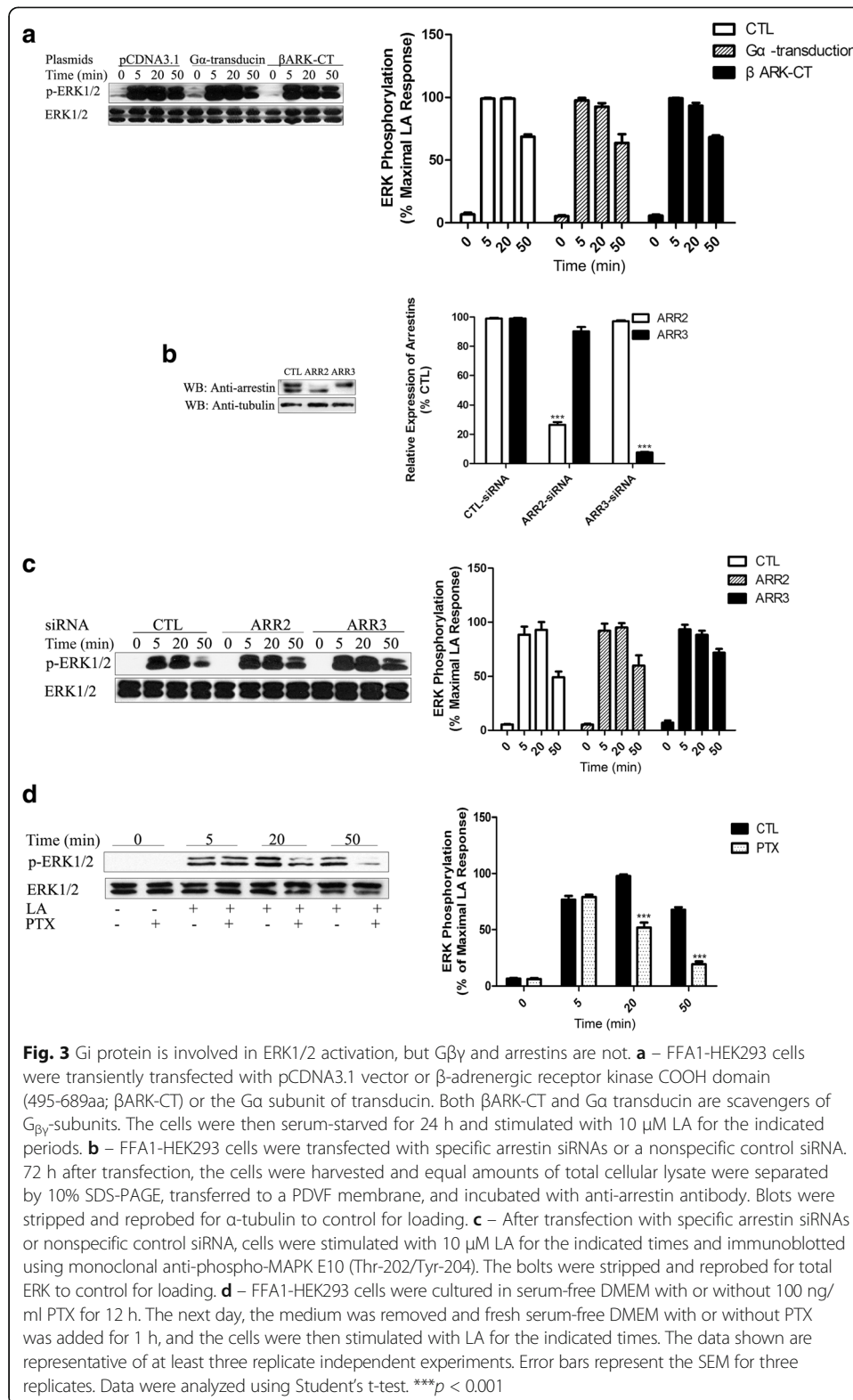


were observed in FFA1-mediated ERK1/2 phosphorylation pre-incubated with the selective Src kinase inhibitor PP2 (Fig. 2c). It is indicated that the non-receptor receptor tyrosine kinase (RTK) Src is not required for FFA1-mediated ERK1/2 activation.

**Gq and Gi protein is involved in ERK1/2 activation without involvement of Gβγ and Arrestins**

The Gβγ subunit of heterotrimeric G proteins has been demonstrated to participate in Gi- and Gq/11-coupled receptor ERK1/2 activation [26]. In this study, we revealed that, upon activation of FFA1 by LA, Gq/11 plays a central role in ERK1/2 activation.

Next, we sought to define the role of the Gβγ subunit in FFA1-induced ERK1/2 phosphorylation. The β-adrenergic receptor kinase COOH domain (amino acids 495–689; βARK1-CT) and the Gα subunit of transducin, both of which are scavengers of the Gβγ-subunits, were transfected into FFA1-HEK293 cells. However, there is no inhibition of the LA-mediated 2 increase/2 increase (Fig. 3a). Recent studies have indicated that β-arrestins function as signal transducers for many GPCRs to mediate ERK1/2 activation [27]. However, the result obtained from siRNA knockdown of arrestins exhibited no inhibition of FFA1-induced ERK1/2 phosphorylation (Figs. 3b and c).



To further confirm the role of Gi in LA-triggered ERK1/2 activation, FFA1-HEK293 cells were cultured in the presence or absence of 100 ng/ml of the Gi protein inhibitor PTX, which prevents the Gi protein from interacting with GPCRs at the cell

membrane. Treatment of FFA1-HEK293 cells with PTX for 12 h did not affect cell viability (Additional file 1: Figure S1). Interestingly, an overnight (12 h) pretreatment with PTX resulted in a significant inhibition of ERK1/2 phosphorylation at late time points ( $\geq 20$  min), whereas the early stage of ERK1/2 increase (at 5 min) was not blocked by PTX incubation (Fig. 3d). This suggests that FFA1 activates the ERK1/2 signaling pathway not only through a Gq-dependent pathway, but a Gi-dependent pathway. Take together, there is no involvement of G $\beta\gamma$  and arrestins in the FFA1 downstream ERK1/2 signal pathway, but Gq and Gi are both involved in regulating late-stage ERK1/2 activation.

## Discussion

FFA1 has been generally accepted to play an important role in various physiological functions. It is recognized as a potential target for the treatment of diabetes, bone remodeling, inflammation and neurogenesis [9, 10, 28]. Signals from almost all GPCRs, including FFA1, activate the ERK signaling cascades, which are associated with variety of cellular processes, including proliferation, migration, survival and apoptosis. Several studies have revealed that in breast cancer cell lines, the ERK1/2 and PI3K pathways are involved in proliferation; in part via FFA1 [29]. In addition, numerous observations suggested FFA1/ERK1/2 activation exhibited anti-apoptotic function [30], supported neuronal survival [10] and memory, induced osteocyte apoptosis [31] and had anti-inflammatory effects such as granule release and gene expression in neutrophils [32]. As a consequence, there is significant interest in understanding ERK1/2 signaling. However, details on the specific signaling pathway and molecular mechanism linking FFA1 to ERK1/2 activation in HEK293 cells remained unclear. We therefore used HEK293 cells that were stably transfected with human FFA1 receptors to characterize FFA1-induced ERK1/2 activation.

HEK293 is a commonly employed cell line used for the molecular and functional characterization of the GPCR signal transduction mechanism. There is growing evidence indicating that the FFA1 downstream ERK1/2 signal pathway participates in the regulation of various physiological functions [10, 30–32]. For better understanding of the specific mechanism of FFA1-mediated ERK1/2 activation, we examined the time course of ERK1/2 phosphorylation. The HEK293 cell lines stably expressing FFA1 showed a time-dependent activation of ERK1/2, peaking at 10 min and returning to the basal level at 60 min.

Fasiglifam previously demonstrated that the activation of FFA1 amplifies glucose-stimulated insulin secretion (GSIS) via IP<sub>3</sub>-induced Ca<sup>2+</sup> oscillations and DAG/PKC-independent Ca<sup>2+</sup> oscillations pathway [24]. We wanted to confirm the role of FFA1 downstream signal pathways in regulating ERK1/2 phosphorylation.

To examine if the dominant pathway for FFA1 to induce ERK1/2 phosphorylation is through G protein coupling, we first explored the role of Gq in the activation of ERK1/2. Upon activation of LA, ERK1/2 activation was significantly attenuated by treatment with the Gq inhibitor UBO-QIC. Our data demonstrated that the FFA1-mediated ERK1/2 activation was inhibited by the PKC inhibitor Go6983, suggesting that the PKC pathway is involved in ERK1/2 activation.

Pancreatic  $\beta$ -cells exhibit Ca<sup>2+</sup> oscillations of variable frequency and amplitude, triggering pulsatile insulin secretion [33]. Therefore, experiments were conducted to



elucidate whether  $\text{Ca}^{2+}$  mobilization of FFA1 has a role in ERK1/2 phosphorylation. Our results showed that FFA1-induced ERK1/2 activation was potently inhibited by the  $\text{Ca}^{2+}$  chelator EGTA and endoplasmic reticulum  $\text{Ca}^{2+}$  ATPase inhibitor thapsigargin at a late time point ( $\geq 20$  min), indicating that Gq or another G protein caused calcium mobilization at a late time point through intracellular calcium release.

An interesting and important observation from our study is that the phosphoinositide-specific phospholipase C (PI-PLC) inhibitor edelfosine significantly blocked GRP40-induced ERK1/2 phosphorylation. PI-PLC is involved in the regulation of a large variety of cellular processes both in the plasma membrane and in the nucleus [34]. Although activation of PI-PLC was evidenced by an increase in the levels of two second messengers, IP<sub>3</sub> and DAG, the nuclear inositide metabolism is regulated independently from that occurring elsewhere in the cell. Additional investigations will be necessary to clarify whether nuclear PI-PLC is involved in the FFA1 downstream signal pathway.

These data show that FFA1 mediates ERK1/2 phosphorylation through dual mechanisms, in which Gq/PI-PLC activate the IP<sub>3</sub>/ $\text{Ca}^{2+}$  and DAG/PKC signal pathways.

Further experiments were performed to examine whether or not EGFR tyrosine kinase, which is another mechanism in ERK1/2 signaling by GPCRs [35], is involved in FFA1. Our results demonstrate that in HEK293 cells, the EGF receptor-selective inhibitor AG1478 and the MMP inhibitor GM6001 do not impair ERK1/2 activation by FFA1 agonists. The Src family non-receptor tyrosine kinases have been proposed as early intermediates in the pathway toward inducing EGF receptor transactivation [36].

In our experiment, the Src-family tyrosine kinase inhibitor PP2 displayed no inhibition upon ERK1/2 activation by n-6-free fatty acid (LA). These results suggest that the transactivation of growth factor receptors does not participate in LA-induced ERK1/2 phosphorylation in HEK293 cells.

Previous studies revealed that as well as activating Gq, upon the administration of agonists, the G<sub>i</sub> subunit can also be combined with FFA1, leading to a change in cell features [21, 22]. It has been reported that G<sub>i</sub>- or Gq-coupled GPCRs activate calcium or ERK1/2 through the G $\beta\gamma$  subunit being released [37, 38]. Numerous distinct mechanisms allow activated GPCRs to signal through the ERK1/2 cascade. However, overexpression of the G $\beta\gamma$  scavenger proteins  $\beta$ ARK-CT or G $\alpha$ -transducin were found to have no effect on ERK1/2 phosphorylation.

Considerable evidence has accumulated implicating arrestins as signal transducers in the mediation of the ERK1/2 cascade [27]. However, knocking down arrestin-2 and -3 expression levels with specific siRNAs showed no effect on FFA1-induced ERK1/2 activation in HEK293 cells. This result suggests that arrestins are unlikely to play a major role in FFA1-mediated ERK1/2 activation.

Interestingly, overnight pretreatment of FFA1 stably expressing HEK293 has been found to significantly reduce ERK1/2 phosphorylation at late time points ( $\geq 20$  min), especially when compared with the degree of increase in ERK1/2 at 5 min. It is conceivable that, upon stimulation by an agonist, FFA1 activates Gq proteins, leading to the activation of PI-PLC. This in turn results in the activation of the IP<sub>3</sub>/ $\text{Ca}^{2+}$  and DAG/PKC signaling pathways through activation of ERK1/2. After 20 min of stimulation by the agonist, the G<sub>i</sub> involved in ERK1/2 phosphorylation, which may activate PI-PLC, leads to extracellular calcium influx. This notion is partially consistent

with other reports demonstrating Gi/Gq-induced synergism in the regulation of intracellular Ca<sup>2+</sup> mobilization and ERK1/2 phosphorylation [39, 40]. However, the Gβγ subunits are not involved in this synergism in the regulation of ERK1/2 activation. Additional investigations will be necessary to clarify how Gi is involved in FFA1-mediated ERK1/2 activation and its physiological function.

## Conclusion

This study provides a detailed delineation of the LA-mediated activation of ERK1/2 in FFA1-HEK293 cells. We propose that upon stimulation of LA, activated FFA1 causes the Gαq/11 protein to activate PI-PLC, causing the IP3/Ca<sup>2+</sup> and DAG/PKC pathways to couple to ERK1/2 phosphorylation, and that there is no MMP/EGFR transactivation pathway involved in ERK1/2 activation. Further experiments confirmed that no arrestins, even Gβγ, are involved in FFA1-induced ERK1/2 activation. Surprisingly, we present evidence that Gi subunits play a critical role in ERK1/2 phosphorylation at late time points (≥ 20 min). These observations may provide new insights into the physiological functions modulated by FFA1-mediated activation of ERK1/2.

## Additional file

**Additional file 1: Figure S1.** Forskolin did not mimic the effect of PTX. A. Serum-starved FFA1-HEK293 cells were pretreated with DMSO or Forskolin (10 μM) for 1 h, and the cells were then stimulated with 10 μM LA for the indicated time. ERK1/2 phosphorylation was assessed by Western blot as described in the Experimental Procedures and corresponding immunoblots were quantified by Bio-Rad Quantity One Imaging system. B. FFA1-HEK293 and HEK293 cells were exposed to PTX(100 ng/ml) for indicated time, and then cell viabilities were evaluated by CCK8 assay at OD450 nm. Error bars represent the SEM for three replicates. The data shown are representative of at least three replicate independent experiments. Data were analyzed using Student's t-test (\* p < 0.001). (DOC 2835 kb)

## Abbreviations

DAG: Diacylglycerol; EGFR: Epidermal growth factor receptor; ERK1/2: Extracellular signal-regulated kinase 1 and 2; IP3: Inositol 1,4,5-triphosphate; LA: Linoleic acid; MMP: Matrix metalloproteinase; PI-PLC: Phosphatidylinositol-specific phospholipase C; PKA: Protein kinase A; PKC: Protein kinase C; PTX: Pertussis toxin

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## Availability of data and materials

Data sharing is not applicable to this article as no datasets were generated or analyzed during this study. Any additional information related to this study is available from the author for correspondence upon reasonable request.

## Authors' contributions

Jing Qian, Yuyang Gu, Chun Wu, Yuqi Chen and Jingmei Zhu conceived and designed the experiments. Xingyi Yao, Chen Bei, Qingqing Zhu and Feng Yu performed the experiments. Jing Qian, Feng Yu and Yuyang Gu analyzed the data and wrote the paper. All authors read and approved the final manuscript.

## Ethics approval and consent to participate

Not applicable.

## Consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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