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Crosstalk between 1,25(OH)$_2$-Vitamin D$_3$ and the growth factors EGF and PDGF-BB: Impact on CYP24A1 expression and cell proliferation

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ABSTRACT

This study explored the signaling interplay between the vitamin D receptor (VDR) and receptor tyrosine kinases (RTKs). Epidermal growth factor (EGF) and platelet-derived growth factor (PDGF)-BB promotes cell proliferation in normal and cancer cells. At the same time, the active form of vitamin D (1,25(OH)$_2$-vitamin D$_3$) inhibits proliferation in some cells. Although EGF receptors (EGFR) and PDGF receptors (PDGFR) activate similar downstream pathways, we found that they interact with VDR signaling in distinct ways. We confirmed that 1,25(OH)$_2$-vitamin D$_3$ induces CYP24A1 gene expression in U2OS, T98G, and U251 cells. We found this to be potentiated when combined with EGF. In contrast, PDGF-BB did not impact 1,25(OH)$_2$-vitamin D$_3$-induced CYP24A1 expression in U2OS cells. The increase in CYP24A1 expression due to the combined action of EGF and 1,25(OH)$_2$-vitamin D$_3$ was dependent on AKT and ERK1/2 activation. Another VDR-responsive gene, CYP27B1, was unaffected by the addition of EGF, suggesting that EGF may have gene-specific effects on VDR signaling. While PDGF-BB did not influence CYP24A1 expression, 1,25(OH)$_2$-vitamin D$_3$ significantly influenced PDGF-BB-induced receptor phosphorylation and cell proliferation. In summary, we found that EGF, but not PDGF-BB, influenced the expression of the VDR-dependent gene CYP24A1, while 1,25(OH)$_2$-vitamin D$_3$ had an inhibitory effect on PDGFR signaling and proliferation. These findings highlight unique crosstalk between 1,25(OH)$_2$-vitamin D$_3$ signaling and EGF or PDGF-BB.

1. Introduction

The active form of vitamin D, 1,25-dihydroxyvitamin D3 (1,25(OH)$_2$-vitamin D$_3$), is a crucial regulator of gene expression via the vitamin D receptor (VDR) [1]. Its primary physiological roles include maintaining calcium homeostasis and supporting bone health [2]. Additionally, research has shown that 1,25(OH)$_2$-vitamin D$_3$ can inhibit cell proliferation and may help suppress cancer progression [3–7]. This effect is partly attributed to VDR's ability to bind and repress the c-Myc promoter, a key driver of growth often deregulated in cancer [8]. 1,25(OH)$_2$-vitamin D$_3$ can also disrupt growth-promoting signal transduction, for example, by downregulating the epidermal growth factor receptor (EGFR) [9–11]. Consistent with these findings, low vitamin D levels have been associated with an increased risk of several cancers, including colon, breast, and prostate cancer [12].

The regulation of 1,25(OH)$_2$-vitamin D$_3$ levels is complex and involves multiple enzymes. CYP27B1 is critical for converting the precursor 25-OH-vitamin D$_3$ into its active form, 1,25(OH)$_2$-vitamin D$_3$, while CYP24A1 catalyzes its breakdown by producing 24-hydroxylated metabolites [1]. Elevated CYP24A1 expression has been linked to advanced-stage prostate cancer, leading to increased degradation of the growth-suppressive 1,25(OH)$_2$-vitamin D$_3$ hormone [13].

VDR is part of the nuclear receptor family and forms a heterodimer with the retinoid X receptor (RXR) to bind DNA response elements [14]. The ability of this complex to regulate gene expression is likely modulated by post-translational modifications triggered by signal transduction pathways from other receptors, as well as the recruitment of co-activator proteins [15].

Receptor tyrosine kinases (RTKs), such as the epidermal growth factor receptor (EGFR) and platelet-derived growth factor receptor (PDGFR), are cell surface receptors that often mediate proliferative signals. Ligand binding to EGFR or PDGFR induces receptor dimerization and *trans*-autophosphorylation of tyrosine residues within their intracellular domain [16]. These phosphorylated tyrosine residues serve as docking sites for intracellular signaling proteins, activating key pathways such as RAS-ERK1/2, PI3K/AKT, PLCγ, and STAT [17].

Crosstalk between nuclear receptors and RTKs can occur through various mechanisms. For instance, nuclear receptors may regulate the

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expression of RTKs or their ligands. In addition, signaling pathways activated by RTKs can induce post-translational modifications of nuclear receptor components, affecting their function. Much research has focused on the estrogen receptor (ER) in this context. For example, HER2-mediated signaling was shown to phosphorylate an ER co-activator, playing a critical role in tamoxifen resistance in breast cancer cells [18]. Additionally, EGF-induced phosphorylation of ER occurs in an ERK1/2-dependent manner [19]. EGF and estrogen have also been found to work additively in enhancing CXCL8 expression, although they likely influence the transcription machinery independently [20]. Another potential mechanism of RTK and nuclear receptor crosstalk is the induction of ligands for the opposing receptor system. In mammary cells, estrogen and progesterone have been shown to promote the expression of the EGFR ligand amphiregulin [21].

While numerous studies have explored the crosstalk between EGFR and ER, likely due to ER's pivotal role in breast cancer progression, much less is known about the interaction between EGFR and the tumor- or growth-suppressive vitamin D receptor (VDR) signaling. However, a study by Dougherty et al. (2014) demonstrated that VDR can inhibit EGFR signaling in colon cancer cells, suggesting a potential growth-suppressive function [22].

Information on the crosstalk between PDGFR and VDR signaling is limited. However, a study by Damera et al. (2009) demonstrated that 1,25(OH)$_2$-vitamin D$_3$ inhibits PDGF-induced DNA synthesis in airway smooth muscle cells [23]. Additionally, autocrine stimulation of PDGF-A in tumors might represent another point of interaction with VDR signaling, as a VDR/RXRα binding sequence essential for transcription has been identified in an enhancer region of the PDGF-A gene [24].

2. Materials and methods

2.1. Chemicals

The following hormones and growth factors were used: 1,25(OH)$_2$-vitamin D$_3$ (sc-202877A, Santa Cruz Biotechnology), EGF (AF-100-15) and PDGF-BB (100-14B), both from Peprotech. The following inhibitors were used: Gefitinib (ZD1839), Selumetinib (AZD6244), AKT inhibitor (MK2206), PI3K inhibitor (LY294002) and p38 MAPK inhibitor (SB203580). In all experiments, the final ethanol and DMSO concentration was kept below 0.1 % (v/v).

2.2. Cell culture and cell treatment

Human osteosarcoma (U2OS) and glioblastoma (T98G, U251) cell lines were cultured in DMEM + GlutaMAX with 10 % FBS. Cells were seeded at 5x10^4 cells/cm^2 and treated with EtOH/DMSO (vehicle), 1,25(OH)$_2$-vitamin D$_3$ (10 nM), PDGF-BB (20 ng/ml), EGF (20 ng/ml), or combinations for 24 h. To assess AKT, MEK1/2, and p38 pathways in CYP24A1 expression, cells were pre-treated with inhibitors of MEK1/2 (AZD6244), PI3K (LY294002), AKT (MK2206) or p38 MAPK (SB203580) for 30 min, followed by 24-h treatments with 1,25(OH)$_2$-vitamin D$_3$ EGF, or combinations.

2.3. RNA extraction and real-time qPCR

Total RNA was extracted with the RNeasy Mini Kit (Qiagen), and 1 μg was reverse transcribed using the qPCRBIO cDNA Synthesis Kit. cDNA was then used for real-time PCR with qPCRBIO SyGreen Mix on a CFX Connect system. β-Actin was used as a control (housekeeping) gene, and each PCR was done and analyzed using the $2^{-\Delta\Delta CT}$ method. All experiments were repeated three times. Primers used were: 5′-TCTACAATGAGCTGCGTGTG-3′ (forward) and 5′-AGCCTGGATAGCAACGTACA-3′ (reverse) for β-actin; 5′-AAACCAGCAGTGAACCCTGT-3′ (forward) and 5′-CACCACACCATACAACAGCTT -3′ (reverse) for CYP24A1; 5′-CAGAGTTGCTATTGGCGGGA-3′ (forward) and 5′- ACACAGAGTGACCAGCGTATTT-3′ (reverse) for CYP27B1; 5′-TCACAGAAGAGCACCCCTGG-3’ (forward) and 5′-GGTGAATAGTGCCTTCCGCT-3’ (reverse) for VDR.

2.4. Western blot

Cells were washed once in cold PBS (14190–169, Thermo Scientific) and lysed in a buffer containing 20 mM Tris HCl pH 8, 137 mM NaCl, 10 % glycerol, 1 % Nonidet P-40, 2 mM EDTA and 0.1 % SDS, supplemented with protease and phosphatase inhibitors (PhosSTOP, 4906837001 and cOmplete, 4693159001, Sigma). The lysates were centrifuged at 12000×g for 15 min and mixed with LDS Sample Buffer with 50 mM dithiothreitol (DTT), denatured at 95 °C for 5 min before loading on Bis-Tris Protein Gel (NP0336BOX, Thermo Scientific) for electrophoresis. Proteins were transferred to PVDF-FL membranes, blocked for 2 h in Intercept blocking buffer (LI-COR Biosciences) and incubated at 4 °C overnight with primary antibodies. The membranes were washed three times for 10 min in 0.05 % Tween-20 in TBS, incubated with secondary antibodies labeled with Alexa Fluor™ 680 or IRDye800 for 1 h, and then washed again. The membranes were then scanned and analyzed using an Odyssey Scanner (LI-COR Biosciences). β-Actin was used as a loading control. The following primary antibodies were used: anti-pY1068-EGFR (#3777), anti-EGFR (#2232), anti-pY857-PDGFRβ (#3166), anti-PDGFRβ (#3169), anti-STAT3 (#9139), anti-pY705-STAT3 (#9145), anti-pS473-AKT (#4060), anti-AKT (#2920), anti-pT202/Y204-ERK1/2 (#9101), anti-ERK1/2 (#4695) from Cell Signaling Technology and diluted 1:1000. Other antibodies used were: Anti-VDR (PP–H4537–00, R&D Systems), anti-PDIA3 (VMA00477, Biorad), anti-β-Actin (sc-47778, Santa Cruz Biotechnology) and anti-β-Actin (ab8227, Abcam)

2.5. Cell proliferation assay

The Click-iT™ EdU Cell Proliferation Kit (Alexa Fluor™ 555) was used to measure proliferation in U2OS cells. After overnight starvation, cells were treated with 1,25(OH)$_2$-vitamin D$_3$, EGF, PDGF-BB, or vehicle control for 24 h, with 10 μM EdU added in the last 4 h. Cells were fixed with 4 % PFA, washed 2x with 3 % BSA in PBS, and permeabilized with TBS with 0.2 % Triton X-100, followed by Click-iT™ Reaction Cocktail incubation. Cell nuclei were stained with Hoechst 33342. Images were acquired with a Zeiss imager M2 microscope equipped with a Plan-Apochromat 63X/1.4 Oil objective, HXP120V light source, Hamamatsu C11440 camera and Zen 2 software. Cube filter sets 43HE and 49 were used (all from Zeiss). The images were analyzed with CellProfiler software v4.1.3. Adjustments of brightness and contrast were made to figure images for visualization purposes.

2.6. Statistical analysis

Statistical analyses were performed with GraphPad Prism 10.0.2 software (GraphPad Software, San Diego, CA, USA). Normality was determined using the Shapiro-Wilk test, and significance was by one-way ANOVA. All data presented were the result of at least three independent experiments. P-values $^{*}<0.05$, $^{**}<0.01$, $^{***}<0.001$ were considered significant.

3. Results and discussion

To investigate potential signaling crosstalk between EGF and 1,25(OH)$_2$-vitamin D$_3$, U2OS osteosarcoma cells were treated with either 1,25(OH)$_2$-vitamin D$_3$, EGF, or a combination of both. We measured the mRNA expression of CYP24A1, a well-established target gene of VDR. As anticipated, 1,25(OH)$_2$-vitamin D$_3$ treatment increased CYP24A1 mRNA levels, whereas EGF alone had no effect (Fig. 1a). Combining 1,25(OH)$_2$-vitamin D$_3$ and EGF boosted CYP24A1 expression compared to 1,25(OH)$_2$-vitamin D$_3$ alone. Similar findings were observed in T98G and U251 glioblastoma cell lines (Fig. 1b and **c**). We examined whether the

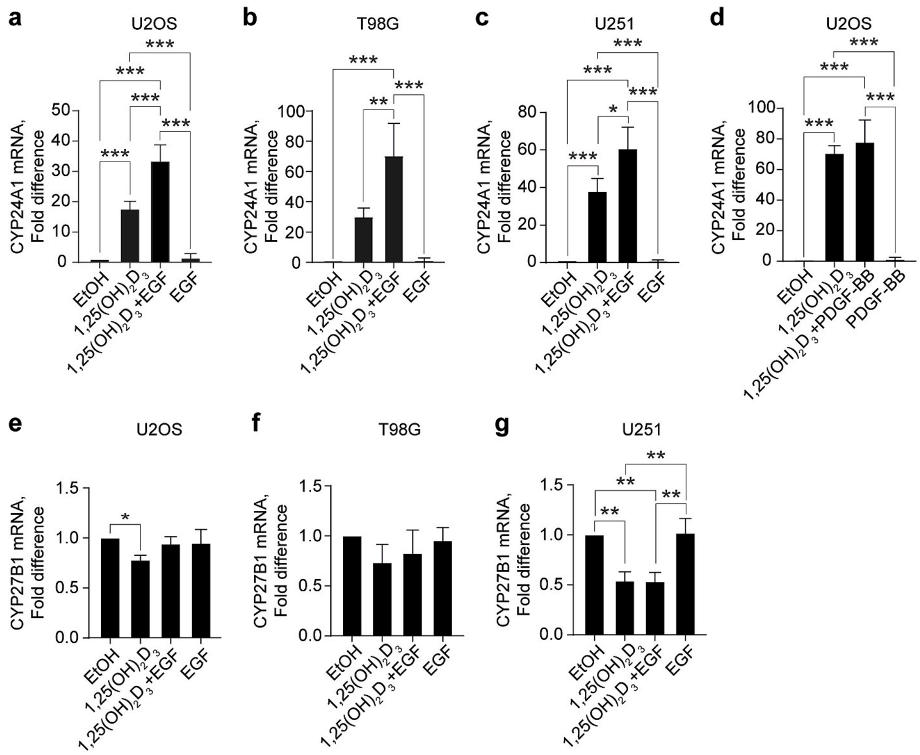

Fig. 1. CYP24A1 mRNA expression is positively upregulated by EGF and $1,25(OH)_2$-vitamin D_3 signaling crosstalk.
The expression levels of CYP24A1 or CYP27B1 mRNA were investigated after 24 h of treatment with either $1,25(OH)_2$-vitamin D_3 (10 nM), EGF (20 ng/ml), or the combination in U2OS- (**a**, **e**), T98G-(**b**, **f**), or U251-cells (**c**, **g**). The effect on CYP24A1 mRNA by PDGF-BB (20 ng/ml) alone or in combination with $1,25(OH)_2$-vitamin D_3 was examined in U2OS cells (**d**). The mRNA expression levels were assayed with RT-PCR and quantified using the $2^{-\Delta\Delta CT}$-method. Fold difference is shown relative to the vehicle control. Error bars represent the standard error (n = 3; ** = $p < 0.01$; *** = $p < 0.001$).

growth factor PDGF-BB influences $1,25(OH)_2$-vitamin D_3-induced CYP24A1 expression similarly to EGF. Interestingly, PDGF-BB did not enhance CYP24A1 expression when combined with $1,25(OH)_2$-vitamin D_3 (Fig. 1d). This indicates that there is selectivity in which growth factors can interact with $1,25(OH)_2$-vitamin D_3 signaling despite the significant overlap in signaling pathways activated by EGFR and PDGFR.

CYP27B1 is another gene regulated by $1,25(OH)_2$-vitamin D_3, although its expression is typically suppressed [25]. Although the effect on CYP27B1 was less pronounced than that observed for CYP24A1, a trend towards suppression was noted in U2OS and T98G cells treated with $1,25(OH)_2$-vitamin D_3, with a more significant response in U251 cells (Fig. 1e, **f** and **g**). In contrast to the findings for CYP24A1, the combination of EGF with $1,25(OH)_2$-vitamin D_3 did not further suppress CYP27B1 expression compared to $1,25(OH)_2$-vitamin D_3 alone. This suggests that while EGF and $1,25(OH)_2$-vitamin D_3 signaling collaboratively enhance the stimulatory VDR response element regulating CYP24A1, they do not similarly affect the inhibitory VDR response elements in the CYP27B1 promoter. Overall, these results indicate that EGF and $1,25(OH)_2$-vitamin D_3 signaling interact within the cell, influencing some (CYP24A1) but not all (CYP27B1) of $1,25(OH)_2$-vitamin D_3-induced changes in gene expression.

Based on these findings, it can be inferred that EGF addition may lead to a reduction in active $1,25(OH)_2$-vitamin D_3 levels. This is because the upregulation of CYP24A1, which EGF induces, contributes to the catabolism of the active form of vitamin D [1]. Furthermore, EGF did not influence the levels of CYP27B1, an enzyme involved in producing biologically active $1,25(OH)_2$-vitamin D_3. The observation that EGF stimulation promotes $1,25(OH)_2$-vitamin D_3 catabolism is consistent with data suggesting an antagonistic relationship between EGFR and VDR [26].

To understand the mechanism behind the increased CYP24A1 expression observed with combined EGF and $1,25(OH)_2$-vitamin D_3 treatment, we examined VDR expression. As shown in Fig. 2a, we did not observe a significant change in VDR mRNA levels with the combined treatment in U2OS cells. Although not statistically significant, there was a slight increase in VDR expression with $1,25(OH)_2$-vitamin D_3 alone, which was not further enhanced by the addition of EGF.

Next, we investigated the role of specific signaling pathways by using low molecular weight inhibitors targeting ERK1/2, AKT, p38 pathways and EGFR itself. As anticipated, inhibiting EGFR kinase activity returned CYP24A1 expression to levels observed with $1,25(OH)_2$-vitamin D_3 treatment alone (Fig. 2b), indicating that the observed synergistic effect on CYP24A1 expression is specific to EGFR signaling. Inhibition of ERK1/2 and PI3K/AKT also reduced CYP24A1 expression to the level seen with $1,25(OH)_2$-vitamin D_3 alone (Fig. 2b). In contrast, blocking p38 signaling did not impact CYP24A1 expression. These findings suggest that PI3K/AKT and ERK1/2 signaling pathways downstream of EGFR are crucial for the enhanced CYP24A1 mRNA expression observed with combined EGF and $1,25(OH)_2$-vitamin D_3 treatment. This result is consistent with previous findings that CYP24A1 induction by fibroblast growth factor 23 is ERK1/2-dependent [27].

To assess the activation levels of AKT and ERK1/2 in U2OS cells treated with EGF, $1,25(OH)_2$-vitamin D_3, or both, we performed immunoblotting using antibodies that detect phosphorylated, active forms of these proteins. As shown in Fig. 2c, EGF treatment for 24 h increased the phosphorylation of EGFR and ERK1/2 compared to vehicle-treated cells. However, $1,25(OH)_2$-vitamin D_3 did not affect the phosphorylation levels of these proteins. AKT was phosphorylated under basal conditions, and its phosphorylation level was unchanged by either treatment (Fig. 2c). These results suggest that while basal activity of the AKT pathway is necessary, it alone is insufficient for the increased CYP24A1 expression observed with EGF and $1,25(OH)_2$-vitamin D_3 co-stimulation. Additionally, EGF increased phosphorylated ERK1/2 levels (Fig. 2c), and inhibition of this pathway diminished the enhanced

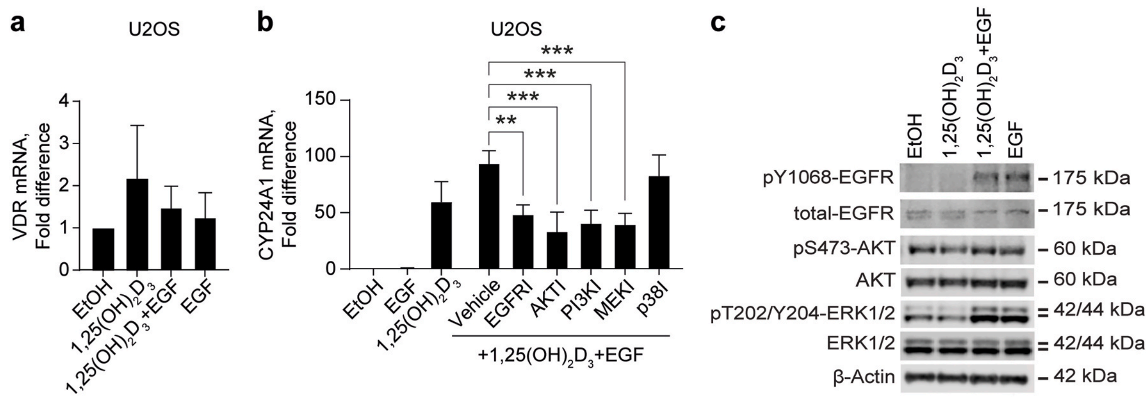

Fig. 2. Activation of the PI3K/AKT and ERK1/2 pathways is required for synergistic increase in CYP24A1 mRNA expression by combined EGF and 1,25 $(OH)_2$-vitamin D_3 treatment.
VDR mRNA levels were measured in U2OS cells after 24-h treatments with $1,25(OH)_2$-vitamin D_3 (10 nM), EGF (20 ng/ml), or the combination (**a**). CYP24A1 mRNA levels were evaluated with or without inhibitors against EGFR (EGFRI, ZD1839, 25 nM), AKT (AKTI, MK2206, 2.5 μM), PI3K (PI3KI, LY294002, 10 μM), MEK1/2 (MEKI, AZD6244, 250 nM) and p38 MAPK (p38I, SB203580, 10 μM) and treatment with either $1,25(OH)_2$-vitamin D_3, EGF, or a combination of both for 24 h (**b**). RT-PCR results were quantified using the $2^{-\Delta\Delta CT}$-method. Fold difference is shown relative to vehicle control. In (**c**), immunoblots with lysates from cells treated with $1,25(OH)_2$-vitamin D_3, EGF, or a combination of both for 24 h, and antibodies targeting total or phosphorylated EGFR, AKT, ERK1/2, are shown. β-actin was used as a loading control. Error bars for all experiments represent the standard deviation (**a-c**, n = 3; ** = $p < 0.01$; *** = $p < 0.001$).

CYP24A1 expression seen with combined EGF and $1,25(OH)_2$-vitamin D_3 treatment (Fig. 2b), compared to treatment with $1,25(OH)_2$-vitamin D_3 alone. Addition of $1,25(OH)_2$-vitamin D_3 did not influence the phosphorylation level of ERK1/2 compared to EGF alone (Fig. 2c).

In a previous study, we demonstrated that silencing expression of the alternative low-affinity $1,25(OH)_2$-vitamin D_3 receptor, PDIA3, resulted in a reduction of $1,25(OH)_2$-vitamin D_3-induced CYP24A1 mRNA levels [28]. To investigate whether PDIA3 might contribute to the increased CYP24A1 expression observed with combined EGF and $1,25(OH)_2$-vitamin D_3 treatment, we analyzed the protein levels of PDIA3 in cells treated with EGF over various time-periods. As shown in Fig. 3a and b, we observed a time-dependent increase in PDIA3 levels in cells treated with EGF. In contrast, PDGF treatment did not influence the expression of PDIA3 (Fig. 3c and d)

Given that $1,25(OH)_2$-vitamin D_3 is known to inhibit cell proliferation [30], while EGF and PDGF-BB promote it [31,32], we investigated their effects on U2OS cell proliferation, both individually and in combination. As anticipated, EGF and PDGF-BB enhanced cell proliferation compared to untreated controls (Fig. 4a and b). The combination of EGF and $1,25(OH)_2$-vitamin D_3 did not significantly alter proliferation compared to EGF alone. However, $1,25(OH)_2$-vitamin D_3 strongly inhibited PDGF-BB-induced proliferation.

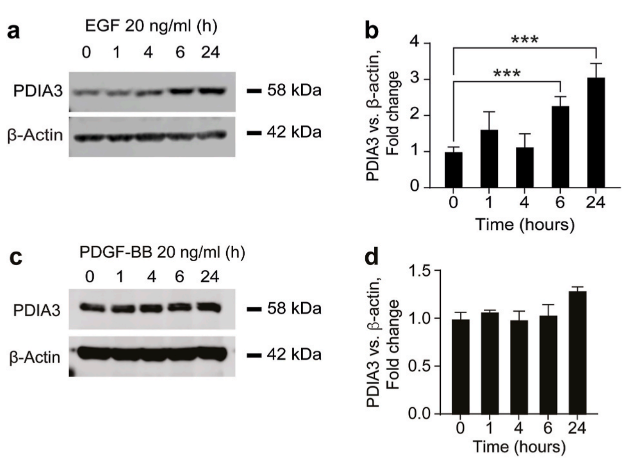

Fig. 3. EGF-signaling upregulates PDIA3 protein expression in U2OS cells.
The cells were treated with EGF (20 ng/ml) (**a, b**) or PDGF-BB (20 ng/ml) (**c, d**) for either 0, 1, 4, 6 or 24 h, after which cell lysates were collected and subjected to SDS-PAGE and transferred to a PVDF membrane. The membrane was immunoblotted with antibodies targeting PDIA3 and β-actin (**a, c**). Quantification of PDIA3 protein expression is shown in (**b, d**). β-actin was used as a loading control. The fold difference was then calculated from the mean of all 0 h replicates. Error bars for all experiments represent the standard deviation and statistical analysis was performed against 0 h control. (**a, b** n = 4; **c, d**, n = 3; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$).

To further explore the mechanism behind this inhibition, we assessed the expression and phosphorylation levels of PDGFRs after 24 h of continuous stimulation. We observed a marked reduction in PDGFR phosphorylation in the presence of $1,25(OH)_2$-vitamin D_3 (Fig. 4c and d). The inhibition of PDGFR phosphorylation by $1,25(OH)_2$-vitamin D_3 likely accounts for the decreased proliferation. The reduction of PDGFR phosphorylation by $1,25(OH)_2$-vitamin D_3 below baseline levels may be due to an autocrine PDGF loop in U2OS cells that maintains high steady-state phosphorylation levels [33]. Additionally, treatment with PDGF-BB resulted in phosphorylation levels comparable to vehicle-treated cells, likely due to 24-h PDGF-BB exposure activating feedback mechanisms that limit receptor phosphorylation. Acute PDGF-BB stimulation, however, caused a sharp increase in PDGFR phosphorylation above the vehicle control (data not shown).

$1,25(OH)_2$-vitamin D_3 has been reported to have roles in angiogenesis, functioning as either a proangiogenic or antiangiogenic agent depending on the specific tissue context [34]. Our results suggest that the effects of $1,25(OH)_2$-vitamin D_3 on angiogenesis may, at least in part, be mediated through its interaction with the PDGF signaling axis. This intersection with PDGF signaling opens an intriguing avenue for further investigation, especially given the extensive research highlighting PDGFR's regulatory role in angiogenesis [17,35].

In summary, our study reveals a specific crosstalk between VDR signaling and the growth factors EGF and PDGF-BB. We found that EGF enhances $1,25(OH)_2$-vitamin D_3 -induced CYP24A1 mRNA expression through AKT- and ERK1/2-dependent mechanisms. In contrast, PDGF-BB does not modulate CYP24A1 expression in response to $1,25(OH)_2$-vitamin D_3. This suggests that EGF, but not PDGF-BB, interacts with VDR signaling to influence CYP24A1 expression. Given that EGF is a well-known mitogenic factor that promotes tumor cell growth, its ability to enhance CYP24A1 expression is consistent with its role in reducing the levels of the growth-inhibitory $1,25(OH)_2$-vitamin D_3, thereby further allowing for EGF-induced cell proliferation. Conversely, we observed no impact of PDGF-BB on VDR-mediated effects. However, $1,25(OH)_2$-vitamin D_3 inhibited PDGFR phosphorylation and negated the mitogenic

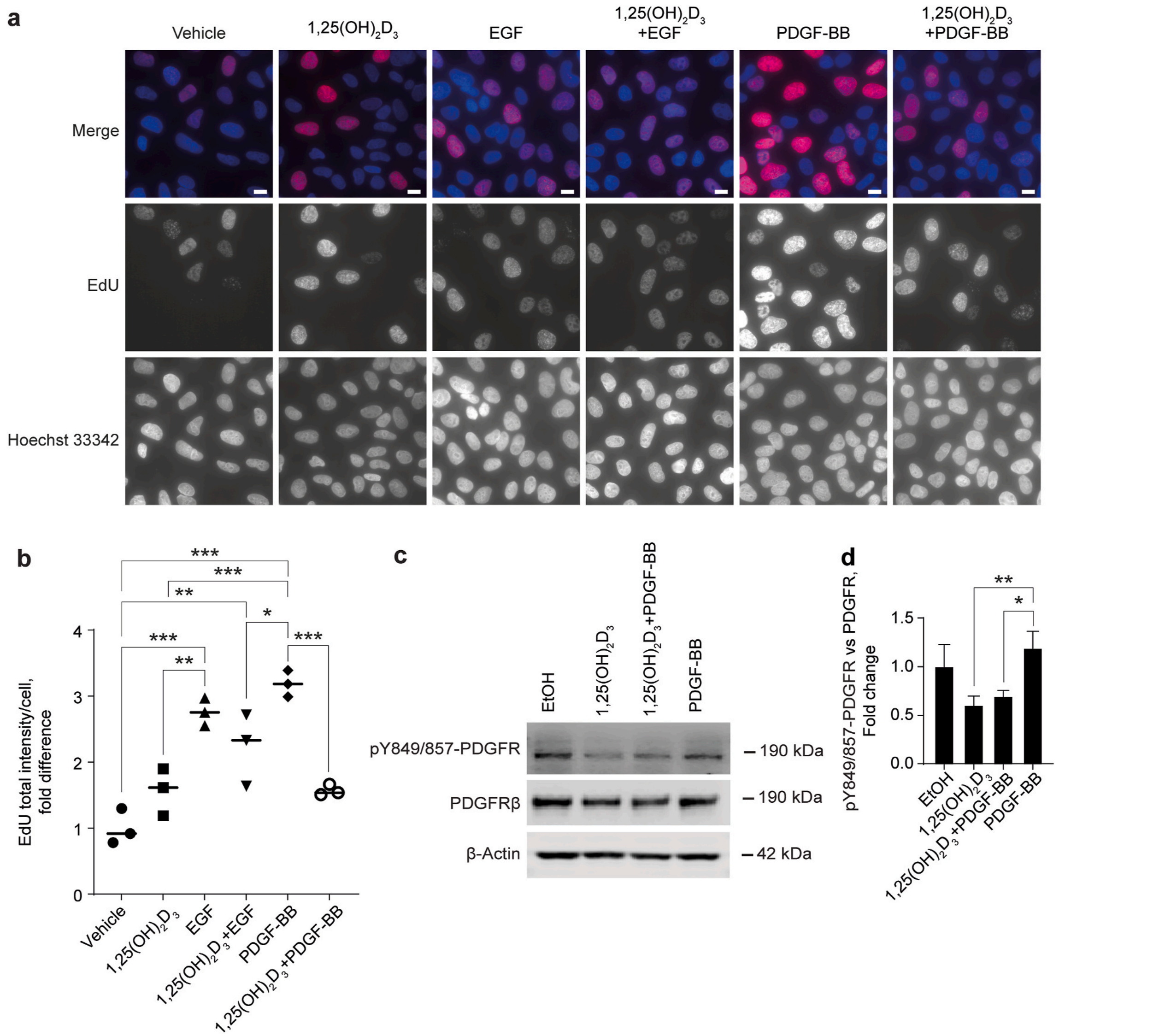

Fig. 4. 1,25(OH)$_2$ vitamin D$_3$ signaling negatively regulates PDGF-BB-induced proliferation in U2OS cells.
Cell proliferation in U2OS cells was assessed using the Click-iT™ EdU Cell Proliferation Kit. Cells were treated for 24 h with 1,25(OH)$_2$-vitamin D$_3$ (10 nM), EGF (20 ng/ml), PDGF-BB (20 ng/ml), or combinations of PDGF-BB or EGF with 1,25(OH)$_2$-vitamin D$_3$. A vehicle control group was also included. EdU (10 μM) was added for the final 4 h of incubation. Panel (**a**) shows representative images where blue indicates nuclei stained with Hoechst 3342, and magenta indicates proliferating cells stained with EdU coupled to Alexa Fluor 594. In panel (**b**), the quantification of proliferating cells is shown, calculated as the relative EdU signal intensity compared to the total number of nuclei in each frame. The fold change was determined by comparing the mean of all vehicle control replicates. In panel (**c**), immunoblots with lysates from cells treated with 1,25(OH)$_2$-vitamin D$_3$ (10 nM), PDGF-BB (20 ng/ml), or both, using antibodies against total or phosphorylated PDGFR-β and β-Actin are shown. In panel (**d**), quantification of pY857-PDGFR levels normalized to total PDGFRβ is shown. Fold changes were calculated relative to the mean of the vehicle control replicates. Scale bar = 20 μm. Error bars represent the standard error (n = 3). Statistical significance is indicated as follows: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

effects of PDGF-BB in U2OS cells. The precise mechanism underlying this inhibition requires further investigation.

CRediT authorship contribution statement

Frida Olsson: Writing – review & editing, Visualization, Validation, Investigation, Formal analysis. **Erik Wåhlén:** Writing – review & editing, Visualization, Validation, Investigation, Formal analysis. **Johan Heldin:** Writing – review & editing, Visualization. **Ola Söderberg:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Maria Norlin:** Writing – review & editing, Supervision. **Johan Lennartsson:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Conflict of interest statement

There is no potential conflict of interest.

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Declaration of competing interest

The authors have nothing to declare.

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