TRPC1 Protein Channel Is Major Regulator of Epidermal Growth Factor Receptor Signaling*

Nicolas Tajeddine1 and Philippe Gailly2

From the Laboratory of Cell Physiology, Institute of Neuroscience, Université Catholique de Louvain, Brussels 1200, Belgium

Background: EGFR regulates cell proliferation and therefore constitute a major target in cancer therapy.

Results: EGF triggers a calcium entry through TRPC1 ion channels, which is crucial to allow complete activation of EGFR and its downstream pathways.

Conclusion: Calcium entry through TRPC1 constitutes an amplification loop in EGFR activation.

Significance: TRPC1 is a potential therapeutic target in cancers resistant to tyrosine kinase inhibitors.

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TRP channels constitute a large family of proteins that are expressed almost ubiquitously. The family was designated TRP because of a spontaneously occurring mutation in Drosophila, the photoreceptors of which lacked TRP and responded to a continuous light with a transient receptor potential. The homologous proteins in mammalian cells seem to mediate responses to agonists, pheromones, odorant ligands, temperature, pH, osmolarity, and oxidative stress (1). However, in contrast with voltage-operated and ligand-gated channels that have been studied in detail, the activation and regulation mechanisms of TRP channels are largely unknown and diverse. Structurally, TRP seems constituted of four subunits having each six transmembrane domains. This is similar to voltage-dependent channels, except that they are not positively charged and usually voltage-insensitive. The TRP channel family is divided in six subfamilies, among which the most important are the canonical TRP subfamily (TRPC1–7), the melastatin-related TRP subfamily (TRPM1–8) and the vanilloid-receptor-related TRP (TRPV1–6) (2). Some of these have been involved in cell proliferation and cancer progression (3). In particular, TRPC1 has been shown to enhance cell proliferation and to regulate cell migration, two processes involved in cancer aggressiveness (4–6).

TRPC1 is a non-selective cation channel (PCa/PM Na~1). It is involved in store-operated calcium entry (also named capacitative entry) in cooperation with Orai1 and activated by STIM1, the sensor of endoplasmic reticulum Ca2+ content (7–10). However, several evidences suggest that TRPC1 can be activated independently of store depletion, but the gating mechanisms are still unknown (11). We previously investigated the mechanisms underlying the role of TRPC1 in cell migration and showed the involvement of calcium-induced activation of calpains and proteolysis of myristoylated alanine-rich protein kinase C substrate, an actin-binding protein possibly involved in focal adhesion (12). Moreover, it has been shown that TRPC1 is a crucial determinant of directionality of migration in response to chemotactic agents (13). Besides, stimulation with epidermal growth factor (EGF) results in TRPC1 channel localization to the leading edge of migrating glioma cells and chemotaxis toward EGF was lost when TRPC1 channel was inhibited (14).

The mechanism underlying the effect of TRPC1 modulation on proliferation remains elusive. Activation of the calcium-sensing receptor by high external calcium ([Ca2+]o) increases cell proliferation and TRPC1 expression (15). Moreover, Ca2+ entry through TRPC1 seems to be involved in the phosphorylation of ERK1/2 upon activation of the Ca2+-sensing receptor (16). Other reports have shown that TRPC1 depletion induced cell growth arrest by blocking the cell cycle in G0/G1 phase in endothelial progenitor cells or by causing incomplete cytokinesis in gliomas (4, 6).

Because TRPC1 is involved in EGF-induced cell migration, we hypothesized that its effect on cell proliferation might be mediated.
by alteration of EGFR signaling. EGFR has been implicated strongly in the biology of human epithelial malignancies, with therapeutic applications in cancers of the colon, head and neck, lung, and pancreas (17). In particular, overexpression or activating mutations of EGFR are found in 40 to 80% of non-small cell lung carcinoma (NSCLC) and associated with poor prognosis, rendering it an attractive therapeutic target (18, 19). Inhibitors of EGFR tyrosine kinase activity are used largely in the treatment of advanced NSCLC (20, 21). However, the efficiency of this therapeutic strategy is limited to a subset of patients who ineluctably develop resistance against currently available EGFR inhibitors such as erlotinib and gefitinib (22). It seems therefore crucial to better understand modulation of EGFR signaling pathways.

In the present study, we evaluated the role of TRPC1 channel in the modulation of EGFR activity in two different cellular models of NSCLC. We show that EGF-induced calcium influx through TRPC1 is an essential event in the triggering of EGFR activity and EGF-induced signaling pathways. Consequently, TRPC1 depletion inhibits cell proliferation. This study provides novel insights in the function of TRP channels in the regulation of signaling pathways involved in cell proliferation.

EXPERIMENTAL PROCEDURES

Cell Culture and Reagents—A549 and H1299 cells (American Type Culture Collection, Manassas, VA) were grown in DMEM/F12 and RPMI1640, respectively, at 37 °C in an humidified atmosphere of 5% CO₂, 95% air supplemented with 10% FCS, 100 IU/ml penicilllin, and 100 μg/ml streptomyacin (Invitrogen). Cells were cultured up to passage 20. Human recombinant EGF and thapsigargin (Sigma-Aldrich) were used at 100 ng/ml and 1 μM, respectively. W-13 was purchased from Tocris (Elisville, MS) and LY294002 and PD98059 from Cell Signaling Technology (Beverly, MA). Xestospongin B was kindly provided by Dr. Jordi Molgo (Gif-sur-Yvette, France).

siRNA Transfection—Depletion of TRPC1 was achieved by using a pool of four siRNAs (called as siTRPC1) targeting four different sequences of human TRPC1 mRNA (5’-GGACUACG-GUUGUCAGAAA-3’; 5’-CGACAAGGGUGACUUAU-3’; 5’-GACUACG-GGUUGUCAGAAA-3’; 5’-CGACAAGGGUGACUUAU-3’).
5'-GUAAGUGAUUGCUCA-3' and 5'-GACGCAAGC-CCACCUGUAA-3'). siTRPC1 as well as the non-silencing control pool of siRNAs (siUNR) were purchased from Thermo Fisher Scientific (Lafayette, CO). A549 and H1299 cells were transfected using DharmaFECT reagent according to the manufacturer's instructions (Thermo Fisher Scientific). Twenty-four hours later, cells were plated on six-well plates or on 10-cm diameter Petri dishes. Cells were analyzed 72 to 96 h after transfection.

Quantitative RT-PCR—A549 and H1299 mRNAs were extracted with Ribopure kit (Ambion, Applied Biosystems, Lennik, Belgium) and reversed-transcribed using SuperScript II RNase H (Invitrogen). Gene-specific PCR primers were designed using Primer3. To avoid amplification of genomic DNA, primers were chosen in different exons. The following primers were purchased from Eurogentec (Seraing, Belgium): 5'-ACTGTGTAGG-CATCTTCTGAAACA-3' (sense) and 5'-GGAGAAAATATA-CCAGAACAAAGCAA-3' (antisense). The β2-microglobulin housekeeping gene and TRPC1 cDNAs were amplified in parallel. Real-time RT-PCR was performed using 5 μl of cDNA, 12.5 μl of SYBRGreen Mix (Bio-Rad) and 300 nM of each primer in a total reaction volume of 25 μl. The reaction was initiated at 95 °C for 3 min, followed by 40 cycles of denaturation at 95 °C for 10 s, annealing at 60 °C for 1 min, and extension at 72 °C for 10 s. Data were recorded on a MyiQ real-time PCR detection system (Bio-Rad), and cycle threshold (Ct) values for each reaction were determined using analytical software from the same manufacturer.

Each cDNA was amplified in duplicate, and Ct values were averaged for each duplicate. The average Ct value for β2-microglobulin was subtracted from the average Ct value for the gene of interest. This ΔCt value obtained in siRNA-TRPC1 or shRNA-TRPC1 silenced myoblasts, or at different stages of differentiation, was then subtracted from the ΔCt value obtained in control conditions (siRNA- or shRNA-treated cells, or at day 0 for the time course) giving a ΔΔCt value. As amplification
efficiencies of the genes of interest and $\beta 2$-microglobulin were comparable, the amount of mRNA, normalized to $\beta 2$-microglobulin, was given by the relation $2^{-\Delta \Delta CT}$.

Flow Cytometry—Cells were counted by flow cytometry. Their volume of distribution was determined by concomitantly detecting fluorescent beads at a known concentration. Cell cycle analysis was performed by permeabilizing fixed cells with 0.01% Triton X-100 and subsequent staining by 50 $\mu$g/ml propidium iodide (PI). Cytofluorometric analyses were performed on a FACSCalibur equipped with CellQuest Pro software (Becton Dickinson).

Double Thymidine Synchronization—Synchronization of A549 and H1299 cells at the G1/S border was performed by adding thymidine (2 mM) to the medium during two consecutive nights.

FIGURE 3. TRPC1 depletion induces cell cycle arrest in G0/G1 phase in H1299 cells. A, DNA content analysis by PI staining in H1299 cells transfected with siUNR or siTRPC1, synchronized by double thymidine blockade and released for 6 h. One histogram is representative of three independent experiments. B, quantification at 0 and 6 h after release. Results are expressed as means ± S.D. (n = 3). *** $p < 0.001$.

FIGURE 4. A, TRPC1 depletion induces repression of cyclines involved in G1/S transition. Immunoblot analysis of cyclins D1, D3, and E 72 or 96 h after transfection of A549 with siUNR or siTRPC1. Data are representative of three independent experiments. B, densitometric quantification of experiment presented in A. Results are expressed as means ± S.D. (n = 3). * $p < 0.05$. A.U., arbitrary units; NS, not significant.
After the first and second overnight culture, the medium was removed, and cells were washed with PBS (3×) and cultured in normal medium in the absence of thymidine. Cell cycle analysis was performed starting after the second removal of thymidine.

Assessment of EGFR Internalization—Assessment of EGFR internalization has been described by Duan et al. (23). Briefly, cells were stained with 100 ng/ml of Alexa Fluor 488-conjugated EGF at 4 °C for 30 min and then rinsed and incubated for...
Role of TRPC1 in EGFR Signaling

TRPC1 Depletion Inhibits Cell Proliferation—We verified by quantitative RT-PCR that TRPC1 channel was by far the most expressed TRPC isoform in A549 and H1299 cells, two models of NSCLC. We used a pool of siRNAs targeting four different sequences of TRPC1 mRNA (referred to as siTRPC1 hereafter) to decrease TRPC1 expression. Immunodetection with an anti-TRPC1 antibody (Epitomics) revealed three bands at 130 kDa, ~130 kDa, and ~70 kDa. As suggested in previous studies, the three bands possibly correspond to multimeric and monomeric forms (25). All of these were decreased significantly 72 and 96 h after siTRPC1 transfection (Fig. 1A). Similar results were obtained with another antibody (provided by Alomone; data not shown). Due to the controversy about the specificity of commercially available TRPC antibodies (26, 27), we confirmed TRPC1 depletion by quantitative RT-PCR. We observed that the content of TRPC1 decreased by 70% 72 h after removal of thymidine, the proportion of siTRPC1-targeted to another TRPC1 mRNA sequence (Fig. 1C). Similar results were obtained with a siRNA sequences of TRPC1 mRNA (referred to as siTRPC1 hereafter) (Fig. 1B). Treating A549 cells with siTRPC1 significantly inhibited cell proliferation with a doubling time of 31.08 h versus 6.5 h in TRPC1 depleted cells versus 23.07 ± 3.5 h in control cells (n = 3, p < 0.05) after siTRPC1 transfection in comparison with cells transfected with an unrelated siRNA (referred to as siUNR hereafter) (Fig. 1B). Treating A549 cells with siTRPC1 significantly inhibited cell proliferation with a doubling time of 31.08 ± 6.5 h in TRPC1 depleted cells versus 23.07 ± 3.5 h in control cells (n = 3, p < 0.05, values calculated on cell quantification at 48 and 72 h) (Fig. 1C). Similar results were obtained with a siRNA targeted to another TRPC1 mRNA sequence (Fig. 1D).

TRPC1 Depletion Induces G₁/G₂ Cell Cycle Arrest—To grossly decipher the mechanism of siTRPC1-induced cell growth inhibition, we analyzed cell cycle by DNA staining and flow cytometry measurements. TRPC1 depletion induced a cell cycle arrest in G₁/G₂ phase in a non-synchronized cell population (Fig. 2, A and C). This effect was made dramatically more visible after double thymidine block (Fig. 2, B and D). Indeed, 6 h after removal of thymidine, the proportion of siTRPC1-transfected cells in G₁/G₂ phase was three times times larger than in control cells (Fig. 2, C and D). More significantly, we observed a significant increase in the proportion of cells targeted to another TRPC1 mRNA sequence (Fig. 1C). Similar results were obtained with a siRNA targeted to another TRPC1 mRNA sequence (Fig. 1D).

RESULTS

TRPC1 Depletion Inhibits Cell Proliferation—We verified by quantitative RT-PCR that TRPC1 channel was by far the most expressed TRPC isoform in A549 and H1299 cells, two models of NSCLC. We used a pool of siRNAs targeting four different sequences of TRPC1 mRNA (referred to as siTRPC1 hereafter) to decrease TRPC1 expression. Immunodetection with an anti-TRPC1 antibody (Epitomics) revealed three bands at 130 kDa, ~130 kDa, and ~70 kDa. As suggested in previous studies, the three bands possibly correspond to multimeric and monomeric forms (25). All of these were decreased significantly 72 and 96 h after siTRPC1 transfection (Fig. 1A). Similar results were obtained with another antibody (provided by Alomone; data not shown). Due to the controversy about the specificity of commercially available TRPC antibodies (26, 27), we confirmed TRPC1 depletion by quantitative RT-PCR. We observed that the content of TRPC1 decreased by 70% 72 h after removal of thymidine, the proportion of siTRPC1-targeted to another TRPC1 mRNA sequence (Fig. 1C). Similar results were obtained with a siRNA sequences of TRPC1 mRNA (referred to as siTRPC1 hereafter) (Fig. 1B). Treating A549 cells with siTRPC1 significantly inhibited cell proliferation with a doubling time of 31.08 ± 6.5 h in TRPC1 depleted cells versus 23.07 ± 3.5 h in control cells (n = 3, p < 0.05) after siTRPC1 transfection in comparison with cells transfected with an unrelated siRNA (referred to as siUNR hereafter) (Fig. 1B). Treating A549 cells with siTRPC1 significantly inhibited cell proliferation with a doubling time of 31.08 ± 6.5 h in TRPC1 depleted cells versus 23.07 ± 3.5 h in control cells (n = 3, p < 0.05, values calculated on cell quantification at 48 and 72 h) (Fig. 1C). Similar results were obtained with a siRNA targeted to another TRPC1 mRNA sequence (Fig. 1D).

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Role of TRPC1 in EGFR Signaling

A

EGF

[B (nM)]

Time (s)

0 100 200 300 400 500 600 700

C

EGF

[B (nM)]

Time (s)

0 100 200 300 400 500 600 700

D

EGF

[B (nM)]

Time (s)

0 100 200 300 400 500 600 700

E

EGF

[B (nM)]

Time (s)

0 100 200 300 400 500 600 700

F

TG 1 µM

[Ca^2+ (nM)]

Time (s)

0 100 200 300 400 500 600 700

G

siUNR

siTRPC1

[Ca^2+ (nM)]

Rest TG SOCE

H

dkDa

p-Akt (S473)
p-p44/p42

β-Actin
Results expressed as means ± S.D. (n = 3). *, p < 0.05; **, p < 0.001. B, immunoblot analysis of phospho-Tyr-1068 EGFR, total (tot) EGFR, phospho-Ser-473 Akt, and total Akt in A549 treated with 100 μM W13 for 24 h or with vehicle alone and stimulated with 100 ng/ml EGF for the indicated times. DMSO, dimethyl sulfoxide.

than in siUNR-transfected cells. Importantly, the effect of siTRPC1 on the cell cycle could be extrapolated to H1299, another NSCLC cell line (Fig. 3).

On the basis of the effects of TRPC1 depletion on the cell cycle, we analyzed the expression of cyclins involved in G1/S transition. Expression of cyclins D1 and D3 was reduced 72 or 96 h after siTRPC1 transfection, consistent with G1/S blockade induced by TRPC1 depletion (Fig. 4).

TRPC1 Mediates EGFR Phosphorylation and Activates EGF-induced Signaling Pathways—As TRPC1 and EGFR cooperate in cell migration, we measured EGFR phosphorylation after TRPC1 depletion in NSCLC cell lines cultured in complete medium (i.e. supplemented with FCS). We observed that phosphorylation on Tyr-1068 was reduced dramatically 72 and 96 h after transfection with siTRPC1 (Fig. 5A). The total amount of EGFR was not modified as shown in immunoblot (Fig. 5A) and quantitative RT-PCR (data not shown). Using FACS analysis of A549 stained with an Alexa Fluor-coupled EGF, we showed that the expression of EGFR at the membrane in basal conditions was unchanged and that kinetics of EGFR internalization was similar in siUNR- and siTRPC1-transfected cells (Fig. 5B). The decreased amount of phosphorylated EGFR protein can therefore be attributed to a decreased activity of the receptor.

We then analyzed the time course of EGFR autophosphorylation in A549 on three different tyrosine residues in response to stimulation with its natural ligand EGF in control conditions and after TRPC1 depletion. For these experiments, FCS was removed 24 h before EGF stimulation. EGF-induced phosphorylation on Tyr-1068 and Tyr-992, two sites involved in downstream signaling pathways, was largely decreased in TRPC1 depleted cells (Fig. 5, C and D). Similarly, we observed that phospho-Tyr-1068 was decreased in H1299 cells transfected with siTRPC1 and treated by EGF (Fig. 5E). In contrast, the ubiquitination triggering site Tyr-1045 was phosphorylated similarly by EGF stimulation in siUNR- and siTRPC1-transfected cells. This is in line with the observation that EGFR expression is not altered by TRPC1 depletion.

TRPC1 Depletion Alters EGFR Downstream Signaling—TRPC1 depletion dramatically decreased activation of two major downstream signaling pathways involved in cell proliferation. Phosphorylation of p44/p42, the major transducer of PI3K action on growth factor-stimulated AGC kinases group to which Akt belongs (28), was decreased after siTRPC1 transfection (Fig. 6). In cells cultured for 24 h in the absence of FCS, Akt, another downstream target of PI3K, was much less phosphorylated in TRPC1-depleted cells in comparison with control cells. However, the acute EGF stimulation seems to overwhelm the effect of siTRPC1 on Akt phosphorylation (Fig. 6). Nevertheless, in proliferating cells cultured in complete medium, siTRPC1 did reduce the amount of phospho-Ser-473 Akt (see Fig. 9). TRPC1 depletion also altered MAPK pathway as demonstrated by the decreased phosphorylation of p44/p42 (Fig. 6).

Calcium Influx through TRPC1 Is Essential for EGFR Activation—As observed previously, EGF induced an oscillatory Ca2+ response in ~50% of A549 cells cultured 24 h without FCS (Fig. 7A). This response was composed of two phases. The first response was dependent on Ca2+ release from internal stores because it was conserved when cells were stimulated with EGF in the absence of external Ca2+. The second phase consisted in repetitive peaks of Ca2+, which were dependent on extracellular Ca2+ (Fig. 7, A and B). Importantly, the second phase was completely abolished in TRPC1 depleted cells (Fig. 7C). To assess that EGF-
triggered Ca\(^{2+}\) entry occurred through TRPC1, we use Alomone blocking antibody to inhibit TRPC1 (29). We observed that second phase of the Ca\(^{2+}\) response to EGF was almost abolished (Fig. 7D).

Pretreatment of A549 cells with the specific of IP3 receptor Xes-tospong B suppressed both Ca\(^{2+}\) release from the endoplasmic reticulum (ER) and late Ca\(^{2+}\) entry from the external medium (Fig. 7E), suggesting that emptying the ER is an obligatory step to allow Ca\(^{2+}\) oscillations observed in the second phase. The release of Ca\(^{2+}\) from the ER could be achieved by thapsigargin, a well known inhibitor of sarco-endoplasmic reticulum Ca\(^{2+}\) ATPase (SERCA) pumps, and triggered a typical store-dependent entry of Ca\(^{2+}\) (Fig. 7F and G). The latter was reduced significantly by TRPC1 depletion. As expected, removal of external Ca\(^{2+}\) and addition of 200 \(\mu\)M EGTA dramatically reduced the activation of PI3K/Akt and MAPK pathways (Fig. 7H).

However, pharmacological inhibition of calmodulin by 100 \(\mu\)M W13 blocked the activation of EGFR and the phosphorylation of one of its downstream target, Akt, demonstrating that EGFR activation was mediated by Ca\(^{2+}\)/calmodulin (Fig. 8A). In agreement, treatment with W13 blocked cell cycle in G0/G1 phase, mimicking the situation observed after TRPC1 depletion (Fig. 8B).

**PI3K/Akt but Not MAPK Is Involved in Cell Cycle Arrest Induced by TRPC1 Depletion**—To further decipher the molecular pathways contributing to the cell growth arrest induced by TRPC1 depletion, we analyzed the involvement of EGFR down-stream targets in A549 proliferation. As expected, inhibiting PI3K with LY294002 and MEK1 with PD98059 respectively inhibited phosphorylation of Akt and p44/p42 MAPK (Fig. 9, A and D). LY294002 induced cell cycle arrest in G0/G1 phase, and reduced cyclin D3 and, to a lesser extent, cyclin D1 expression (Fig. 9B and C). Importantly, siTRPC1 inhibited Akt phosphorylation independently of cell cycle progression (Fig. 9G). In contrast, PD98059 was almost inefficient in altering cell cycle pro-
gression and cyclins D1 and D3 expression (Fig. 9, E and F). The effect of TRPC1 depletion on phosphorylation of p44/p42 was finally studied on synchronized cells. We observed that it induced a delay of phosphorylation (maximum observed 6 h after removal of thymidine block instead of 4 h in control cells). This is most likely a consequence of a reduced rate of cell cycle progression. Indeed, variation in phosphorylation status of p44/p42 during the cell cycle has been documented previously (30).

DISCUSSION

EGFR is a transmembrane tyrosine kinase that belongs to the human epidermal growth factor receptor/ErbB protein family. Its mechanism of activation relies on receptor dimerization and autophosphorylation (31). The Grb2 and Gab1 adaptor proteins bind EGFR at phospho-Tyr-1068 and phospho-Tyr-1086 and lead to the activation of the MAPK and PI3K/Akt pathways (32, 33). PI3K stimulates synthesis of PIP3 from PIP2. PIP3 activates Akt directly or indirectly via PDK1. Phospholipase C binds at phospho-Tyr-992, resulting in its activation (34). Receptor ubiquitination and degradation are the consequences of EGFR phosphorylation at Tyr-1045 (35).

Pioneer studies showed that addition of EGF causes an increase in cytoplasmic free calcium concentration ([Ca2+]i), which completely depends on extracellular Ca2+ (36, 37). More recently however, several studies showed that EGF induced complex oscillatory changes in [Ca2+]i, due to both a release of Ca2+ from the endoplasmic reticulum and a Ca2+ influx from the outer medium (for review, see Ref. 38). Conversely, Ca2+-calmodulin complex regulates EGFR activity either directly or via calmodulin-dependent kinases. Indeed, binding of the calcium-calmodulin complex to EGFR allows its activation by EGF, whereas phosphorylation of EGFR on Ser-1046 and Ser-1047 by calmodulin-dependent kinases decreases its tyrosine kinase activity and increases its rate of internalization.

The major novelty of our study is to point out the TRPC1-mediated Ca2+ regulation of EGFR. We clearly demonstrate that Ca2+ entry through TRPC1 is an obligatory step to allow complete activation of EGFR and its downstream targets in A549 and H1299, two NSCLC cell lines. This effect is mediated by calmodulin because its pharmacological inhibition abolishes EGFR activation. It results in a strong inhibition of G1 to S transition. Interestingly, the MAPK pathway seems to have a marginal effect on A549 cell cycle progression. In contrast, pharmacological inhibition of the PI3K/Akt pathway strongly blocks cell cycle. This effect is associated with a decreased expression of cyclins D1 and D3. This is compatible with the fact that pAkt increases cyclin D1 and D3 expression, including in NSCLC (39, 40). We therefore suggest that the previously reported anti-proliferative effect of TRPC1 blockade might due to EGFR signaling disruption (Fig. 10).

Our results also show that EGF stimulation induces a first [Ca2+]i transient due to Ca2+ release from the ER, followed by an oscillatory [Ca2+]i response dependent on external Ca2+.
Role of TRPC1 in EGFR Signaling

The first phase can be attributed to phospholipase C-mediated synthesis of IP₃ because xestospongin B completely abolishes Ca²⁺ transients in response to EGF. The second phase is mediated by TRPC1 activation because it is abolished after TRPC1 depletion. The exact mechanism of EGF-mediated TRPC1 activation remains elusive but could be, at least partially, of capacitative nature. Indeed, it is dependent on IP₃-mediated Ca²⁺ release, and TRPC1 depletion inhibits capacitative Ca²⁺ entry evoked by thapsigargin. We cannot exclude that ER depletion exerts a permissive effect on the process and that EGFR directly or indirectly activates TRPC1. Indeed, TRPC1 has been reported to be directly activated by PKC and by phosphorylation by PKC (41, 42). TRPC1 is also able to cluster with stimulated growth factor receptors (43). Altogether, our data suggest that Ca²⁺ entry through TRPC1 constitutes an amplification loop: it is triggered by EGFR stimulation, and conversely, it enhances EGFR autophosphorylation and activity.

Recently, several TRP channels, including TRPC1, have been associated with proliferative phenotype in breast cancer (44). This correlation could be explained by our results about the effect of TRPC1 expression on EGFR signaling.

In conclusion, our study points out TRPC1 as a major regulator of EGFR signaling and makes TRPC1 an interesting target in the management of NSCLC patients.

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Role of TRPC1 in EGFR Signaling

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