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Research Article

The Conductance In Between By Activating Estrogen Receptors, the Ca2+-Activated K+ Channel Inhibitor TRAM-34 Promotes the Growth of Breast Cancer Cells

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Abstract

Context and goal: K+ channels contribute to the growth of cancer cells. We have looked into how some K+ channel inhibitors affect the growth of breast cancer cells both at rest and in response to estrogen.

Method of experimentation: We measured cell proliferation by radiolabelled thymidine incorporation using the mammary adenocarcinoma cell line MCF-7, either in the presence or absence of several K+ channel inhibitors, and with or without 17b-oestradiol.

Important outcomes: Kv10.1 and KCa3.1 K+ channel inhibitors inhibited MCF-7 cell basal proliferation, but not estrogen-stimulated proliferation. Depending on the concentration, TRAM-34, a particular inhibitor of KCa3.1 channels, either enhanced or decreased cell growth. TRAM-34 promoted cell proliferation at intermediate concentrations (3 mM-10 mM), but inhibited cell growth at higher values (20 mM-100 mM). Tamoxifen and ICI182, 780, two estrogen receptor antagonists, inhibited the boost of cell proliferation brought on by TRAM-34. Moreover, TRAM-34 raised progesterone receptor mRNA resin, lowered the expression of the estrogen receptor-amRNA, and decreased the amount of radiolabelled estrogen that bound to the MCF-7 estrogen receptor, all of which were similar to the effects of 17b-oestradiol.

Inferences and conclusions: Our findings show that not estrogen-stimulated MCF-7 cell proliferation is basal, but that K+ channels Kv10.1 and KCa3.1 are involved in it. TRAM-34 directly interacts with the estrogen receptor and imitates the effects of 17b-oestradiol on MCF-7 cell proliferation and gene regulation in addition to blocking KCa3.1. Our discovery that TRAM-34 can activate the estrogen receptor raises questions about how to interpret its use and proposes a novel activity for this purportedly selective K+ channel inhibitor.

Keywords: TRAM-34; Estrogen; Potassium channel; Proliferation; MCF-7; Cancer

Abbreviations

CPM: Counts Per Minute; DCC: Dextran-Coated Charcoal; dNTP: Deoxynucleotide Triphosphate; E2: 17b-Oestradiol; ER: Estrogen Receptor; FBS: Fetal Bovine Serum; HAP: Hydroxyapatite; HPRT: Hypoxanthine Phosphor Ribosyl Transferase

Introduction

Potassium channels are crucial for many different biological processes. Specific K+ channels have been shown to play key roles not only in electrical excitability and cell signaling [1], but also in the proliferation of both malignant and normal cells [2-4]. When comparing cancer cells to normal cells from the same region, aberrant expression of K+ channels has been shown [2]. Aberrantly expressed K+ channels have been proposed as novel targets for cancer therapy [5,6] and as biomarkers for the identification of cancer cells [7,8]. The reduction of specific K+ channel inhibition according to Parado et al. (2005) and Ouadid-Ahidouch and Ahidouch (2008) [4,9], including breast cancer cells. Although the exact relationship between K+ channel activity and cell proliferation is unknown, it has been proposed that this activity is critical for the progression of the cell cycle because it regulates the resting membrane potential. This concept states that K+ channel blockage depolarizes the membrane potential, which prevents proliferation [10,9]. Mammary adenocarcinoma cell line MCF-7 is frequently used to investigate the pathogenesis of breast cancer. The mRNAs for many K+ channels, including voltage-gated [KCNQ1 [11], KCNH1 [12], and KCNH2 [13], are expressed by MCF-7 cells (channel nomenclature follows Alexander et al., 2008) [14] and K+ triggered by calcium [15] used KCN4 channels, while [16] used KCNMA1 channels. For the majority of these K+ channels found in MCF-7 cells, channel activity has also been seen [11,12,15,16]. Furthermore, it has been shown that specific K+ channels exhibit cell cycle fluctuations in both expression and channel activity [12,15,16]. The role of certain K+ channels in MCF-7 cell growth has been shown using both molecular (agents for mRNA knockdown) and pharmacological (channel inhibitors) methods. For instance, according to Ouadid-Ahidouch et al. (2004) [15], clotrimazole has been used to link KCa3.1 (KCNN4) to cell proliferation, whereas astemizole or imi-pramine have been used to link Kv10.1 (KCNH1) to same procedure [13]. Moreover, the contribution of this channel to MCF-7 cell proliferation has recently been verified by small interfering RNA targeting Kv10.1 [17]. The onset and spread of breast cancer are significantly influenced by estrogens [18]. Both healthy and malignant mammary gland cells that express the proper receptor-oestrogen receptor-(ER)-a or b-proliferate more when exposed to

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estrogens. Not surprise, MCF-7 cells treated with estrogens multiply substantially quicker. These cells express both ER-a [19] and ER-b [20]. It has recently been shown that some K+ channels support the growth factors' proliferative effects on MCF-7 cells [21,22]. We hence aimed to ascertain the proportionate share of various particular K+ channels in the estrogen-induced proliferation of MCF-7 cells. First, we verified the mRNA expression of particular K+ channels, such as KCNH1, KCNH2, KCNJ8, KCNMA1, KCNN4, and KCNQ1, using RT-PCR. The contribution of each K+ channel type to basal and estrogen-stimulated proliferation was next ascertained by employing particular channel inhibitors. A unique biphasic response was produced by one particular channel inhibitor, TRAM-34, which blocks KCa3.1 (KCNN4) and, depending on its dosage, either stimulates or inhibits growth. As a result, we also looked into other potential mechanisms by which TRAM-34 may affect MCF-7 cell proliferation.

Methods

Culture of cells

The American Tissue Culture Collection (Rockville, MD, USA) provided the MCF-7 cells. Minimal Essential Medium (MEM) supplemented with 10 mM non-essential amino acids, 100 U•mL-1 penicillin, 100 mg•mL-1 streptomycin (all from Invitrogen, Burlington, ON, Canada), and 100 mg•mL-1 human insulin (Sigma-Aldrich, Oakville, ON, Canada) was used to maintain stock cultures. The culture medium was kept humid at 37°C with 5% CO2. Five ÷ 104 cells were placed in each well of a six-well plate for mRNA expression (detection and quantitative PCR) and cell counting. A 24-well plate was used, with 2.5 • 104 cells planted in each well for the incorporation of [3H]-thymidine. TRIzolTM reagent (Invitrogen) was used to extract MCF-7 total RNA for Reverse Transcription (RT), PCR, and RNA extraction after the manufacturer's guidelines. Following that, any contaminating genomic DNA was eliminated using the Turbo DNA-freeTM Kit (Ambion, Austin, TX, USA). Reverse transcription of 2 mg of RNA was carried out in 100 mL of total volume using 5 mM deoxynucleotide Triphosphate (dNTP) mix (Invitrogen), 1 mM oligo (dT) (GE Healthcare Life Sciences, Baie d'Urfé, PQ, Canada), and 200 U of M-MLV reverse transcriptase enzyme (Invitrogen). Each of the Invitrogen primers was created to amplify exon-exon junctions, and by conducting a negative RT reaction without the M-MLV reverse transcriptase enzyme, the absence of genomic DNA amplification was verified. Provides primer sequences, annealing temperatures, and anticipated amplicon sizes. The following were the PCR conditions: forty rounds of denaturation at 95°C after a first melting phase lasting one minute at 95°C for 45s, a suitable temperature for annealing for 45s, and one minute of extension at 72°C. After that, the PCR was extended one last time for 10 minutes at 72°C, then it was stored at 4°C. The following chemicals were used in each PCR reaction, totaling 25 milliliters: 100 pM of the matching forward and reverse primers, 200 mM dNTP mix (Invitrogen), 1.5 mM MgCl2 (Fermentas), 2.5 U Taq DNA polymerase (Fermentas), and 2 mL of cDNA are needed for the PCR. To verify identity, PCR amplicons were extracted, gel electrophoresis was used to visualize them, and a commercial sequencing laboratory (DalGEN Microbial Genomics Centre, Dalhousie University, Halifax, NS, Canada) sequenced them. Utilizing the Roche LightCy-cler® system (Roche Applied Sciences), quantitative RT-PCR (qRT-PCR) Science, Laval, PQ, Canada) in order to identify and measure alterations in gene expression, following the previously mentioned protocol [23]. Progesterone Receptor (PR) and ER-a transcript levels were represented as a percentage of control for each trial, and normalized to the level of the housekeeping gene, Hypoxanthine Phosphoribosyl Transferase (HPRT) bought from Sigma-Aldrich. The supplier of ICI182, 780 (ICI) was Tocris Bioscience, located in Ellisville, Missouri, USA. All medications were diluted and dissolved in the proper vehicles in accordance with the manufacturer's recommendations. Dimethyl sulfoxide (vehicle) concentration for proliferation experiments was not higher than 0.7% v/v. A Trypan blue exclusion assay was used to establish that a single high concentration of channel blockers was safe to use [3H]-The addition of thymidine. The measurement of MCF-7. The process of cell growth was followed as previously stated [13]. In a nutshell, MCF-7 cells were first cultivated in standard medium supplemented with 5% FBS. 5% dextran-coated charcoal-FBS (DCC-FBS, Hyclone, Logan, UT, USA) in phenol red-free MEM (Invitrogen) supplemented with supplements was added to the media after a 24-hour period. The cells were kept in 1% DCC-FBS phenol red-free MEM with supplements for 48 hours after being partially synchronized with respect to the cell cycle after 72 hours. Following synchronization, cells were treated for 36-48 hours with the specified medication in 5% DCC-FBS phenol red-free MEM including supplements together with 1 mM non-radioactive thymidine and [methyl-3H]-thymidine (TRK-300, GE Healthcare Bio-Sciences Inc.; 1 mCi•mL-1). As previously mentioned [13], DNA was extracted, and radioactivity was measured on a count per minute (cpm) for the Beckman LS 5000TA scintillation counter (Beckman Coulter Canada, Mississauga, ON, Canada). Every treatment was carried out on six different wells, and the cpm values were translated onto the same plate into a percentage of the control values. Cell count in MCF-7 cells were cultivated similarly to [3H]-thymidine incorporation (see above), with the exception that the cells were treated with the prescribed medication for 72 hours after synchronization in 5% DCC-FBS phenol red-free MEM including supplements. A Coulter Counter® model ZM30383 (Beckman Coulter ER competitive binding assay) was used to count the diluted cell solution after the cells were disintegrated using 1 mL of 0.25% trypsin/EDTA (Invitro-gen). The MCF-7 ER protein collection process was completed in accordance with earlier reports [24]. In short, MCF-7 cells were cultured in 5% FBS MEM with supplements for four days after plating into 10 cm dishes. Afterwards, the medium was switched to 5% DCC-FBS phenol red-free MEM with nutrients, and the cells were cultivated to 85% confluence. After that, the cells were suspended and incubated for 30 minutes at 37°C in phosphate-buffered saline containing 1 mM EDTA Ca2+-Mg2+. Multiple plates' worth of cells were mixed, centrifuged at 1000 g for five minutes, and then disturbed with ice-cold water, pH 7.4 (TEDG buffer) with HALTTM Protease-inhibitor Cocktail (Fisher Scientific, Ottawa, ON, Canada) and disrupted by ultrasonification contains 10 mM TRIS, 1.5 mM EDTA, 1 mM dithiothreitol, and 10% glycerol. To extract cytosolic protein, the cell lysate was centrifuged at 100,000 ¥g for 30 minutes at 4°C, then collecting the supernatant. The Bradford method (Bio-Rad, Hercules, CA, USA) was used to calculate the protein concentrations, and 500 mL aliquots were kept at -80°C. The following competitive binding experiments were carried out. 250 mg of MCF-7 cell protein was treated in TEDG buffer at room temperature for two hours while containing 0.1 nM [2,4,6,7,16,17-3H(N)]. –oestradiol ([3H]-E2) in a final volume of 500 mL (110 Ci•mmol-1; Perkin-Elmer, Waltham, MA, USA). When non-radioactive E2 was present in an excess of 100 times, non-specific binding was evaluated. Before being introduced to the cytosolic protein, TRAM-34 and E2 standards were diluted in phenol red-free 5% DCC-FBS MEM containing supplements. A vehicle control that included supplements with 0.7% DMSO and 5% DCC-FBS MEM. ToTo distinguish between

ER-bound and unbound [3H]-E2, 250 mL of hydroxylapatite (HAP, 60% in TEDG buffer, Sigma-Aldrich) was added. The mixture was centrifuged at 1000 g for 10 minutes and vortexed every 5 minutes for 15 minutes. The HAP-[3H]-E2-ER complex underwent a wash step using TEDG buffer, centrifugation, and repeating the process. 500 mL of 100% ethanol was added, and the mixture was centrifuged at 1034 ¥g for 10 minutes after being incubated for 15 minutes to extract [3H]-E2 from the HAP-[3H]-E2-ER complex. Once extracted, the separated [3H]-E2 was mixed with 2 ml of scintillation fluid. A Beckman LS 5000TA scintillation counter (Beckman Coulter Canada) was used to measure radioactivity. The competition between TRAM-34 and [3H]-E2 was measured four times in duplicate using four different protein extraction methods. A seeming separation Scatchard analysis yielded a maximal binding capacity of 48.3 ± 5.4 fmol•mg-1 (n=3) and a constant of 0.135 ± 0.034 nM (n=3). Analytical statistics Student's t-test was used to analyze the data for the [3H]-thymidine incorporation studies where there were no paired values. ANOVA was used in conjunction with Tukey's post hoc test to analyze data related to cell counting, qRT-PCR, and competitive binding. Prior to normalization for qRT-PCR and [3H]-thymidine incorporation, data were analyzed. P-values less than 0.05 were regarded as significant.

Result

K+ channel involvement in MCF-7 proliferation RT-PCR was carried out on MCF-7 total RNA to determine the mRNA expression of particular K+ channels in MCF-7 cells The PCR products with lengths of 336, 154, 177, 172, 158, and 153 bp were amplified by primers designed for KCNJ8 (RefSeq: NM-004982), KCNQ1 (NM-000218.2, NM-181798 and NM-181797), KCNH1 (NM-172362 and NM-002238), KCNH2 (NM-000238, NM-172056 and NM-172057), KCNN4 (NM-002250), and KCNMA1 (NM-001014797 and NM-002247). After being removed from the agarose gel, each amplicon was sequenced to ensure that the projected sequence was accurate (data not shown). Thus, Figure 1 verifies that our MCF-7 cells express KCNQ1 mRNA transcripts, which is in line with other findings [11-13,15,16]. Nevertheless, we have additionally shown that mRNA transcripts for the ATP-sensitive K+ channel KCNJ8 are also expressed by MCF-7 cells. To ascertain the relative contributions of each of these K+ channel types to the proliferation of MCF-7 cells, specific channel blockers were employed in conjunction with the [3H]-thymidine incorporation technique to evaluate DNA synthesis. Astemizole for Kv10.1 (KCNH1), E-4031 for Kv11.1 (KCNH2), clotrimazole for KCa3.1 (KCNN4), iberiotoxin for KCa1.1 (KCNMA1), and chromanol 293B (293B) for Kv7.1 (KCNQ1) were all blocked by glibenclamide. The aggregated normalized results from trials 3-11 are displayed in Figure 1. The only drugs that significantly affected proliferation were astemizole and clotrimazole, both of which led to a reduction. As previously reported (Roy et al., 2012), astemizole (3 mM, n=11) reduced [3H]-thymidine incorporation by approximately 50% when compared with controls (P<0.05).2008), but [3H]-thymidine incorporation was reduced by about 20% when clotrimazole (10 mM, n=6) was used in comparison to controls (P<0.05). [3H]-thymidine incorporation was unaffected by gliberetinoin (30 nM, n=4), chromanol 293B (100 mM, n=3), E-4031 (3 mM, n=5), and iberiotoxin (100 nM, n=3). Therefore, these observations support earlier theories [12,13,16] that MCF-7 cell proliferation is influenced by Kv10.1 and KCa3.1 channel activity. On the other hand, there is no proof that the K+ channels Kir6.1, Kv7.1, Kv11.1, or KCa1.1 promote cell division. Next, we looked into the possibility that Kv10.1 and KCa3.1 channel activity plays a role in the proliferation triggered by estrogen displays pooled K+ channel data blockers used in combination or alone (1 nM) E2. In every experiment, E2 by itself led to an approximately 60% increase in [3H]-thymidine incorporation when compared to controls (n=28, P<0.05). The presence of E2 was able to significantly stimulate [3H]-thymidine incorporation in the presence of astemizole (P<0.05), despite the fact that astemizole (a Kv10.1 inhibitor, 3 mM) itself reduced [3H]-thymidine incorporation by around 50%. The same outcome was seen with clotrimazole (KCa3.1 inhibitor); at 10 mM, this medication greatly reduced the incorporation of [3H]-thymidine by approximately 20%; yet, the addition of E2 (P<0.05) boosted the incorporation of [3H]-thymidine. Astemizole and clotrimazole suppression of basal proliferation suggests that Kv10.1 and KCa3.1 channels are involved in constitutive processes that regulate cell growth. Still, an intact response to E2 was demonstrated by a continuous increase of approximately 50% in the presence of astemizole or clotrimazole. The comparable increases in [3H]-thymidine incorporation (~50%-60%) seen with E2 in the presence or absence of both inhibitors show that estrogens' capacity to promote MCF-7 cell proliferation was unaffected by the pharmacological inhibition of Kv10.1 or KCa3.1 activity. Therefore, while not necessary for oestrogen-stimulated proliferation, Kv10.1 and KCa3.1 channels may support basal MCF-7 proliferation. Results with TRAM-34, another KCa3.1 channel blocker that is thought to be more selective than clotrimazole, were significantly different. As a result, 10 mM TRAM-34 surprisingly raised [3H]-thymidine incorporation by around 50% when compared to controls (P<0.05) and stopped E2 from causing any additional increase.

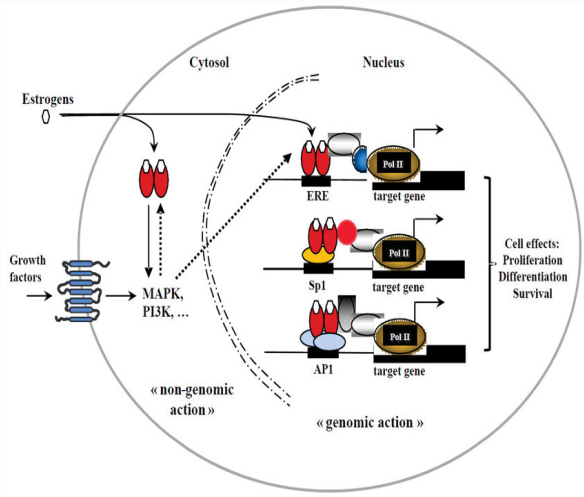

Figure 1: Structure and mechanisms of action of the Estrogen Receptor (ER). (A) The evolutionarily conserved domains of several nuclear receptors, including ER, AR (Androgen Receptor), PR (Progesterone Receptor), GR (Glucocorticoid Receptor), VDR (Vitamin D Receptor), RAR (Retinoid Acid Receptor) and TR (Thyroid Receptor). Domains involved in DNA and ligand binding, as well as in dimerization, ligand-independent transactivation function (AF1) and ligand-dependent transactivation function (AF2) are shown. The number of amino acids for each domain is presented. The approximate molecular weight of each nuclear receptor is also indicated on the right side; (B) Estradiol (E2) mediates multiple phenotypic changes in cells by binding to its receptor. E2 enter the cell through the lipid membranes and binds ER in the cytoplasm or the nucleus. ER mediates E2 effects through diverse transcriptional mechanisms. In the nucleus, the activated ER forms a dimer to tightly fix DNA directly at the ERE sites or indirectly at Sp1 or Ap1 sites. The activated ER is then able to recruit cofactors and RNA polymerase II (pol. II), which allows the transcription of target genes (ER genomic action). Furthermore, ERs can use rapid non-genomic action through the activation of intracellular kinases related or not to the growth factor signaling.

Discussion

K+ channels and the growth of MCF-7 cells

It has been demonstrated that MCF-7 cell proliferation is influenced by the activity of particular K+ channel types [4,9]. MCF-7 cell growth is inhibited by pharmacological drugs that block Kv10.1 and KCa3.1 [13,15]. Our findings, which we conducted using astemizole, clotrimazole, and TRAM-34, support earlier reports that suggested Kv10.1 and KCa3.1 contribute to basal MCF-7 cell proliferation. However, our data also suggest that several other K+ channels expressed in these cells (Kir6.1, Kv7.1, Kv11.1, and KCa1.1) are not likely to be important for basal cell proliferation. We further propose that oestrogen-stimulated cell proliferation can occur without Kv10.1 and KCa3.1 channels of insulin-like growth factor-1 in MCF-7 cells, which has been shown to be regulated by the Akt signaling pathway and dependent on the activity of Kv10.1 channels [22]. An innovative ER agonist is TRAM-34. From a group of prepared clotrimazole derivatives, TRAM-34 was chosen due to its high affinity for KCa3.1 channels (Kd=20 ± 3 nM) and lack of cytochrome P450 inhibition. TRAM-34 was initially developed as a derivative of the commonly used KCa3.1 channel blocker clotrimazole [25]. The structural difference between TRAM-34 and clotrimazole is the substitution of a pyrazole ring for the imidazole ring. TRAM-34 is often utilized to show that KCa3.1 has a function in cells of insulin-like growth factor-1 in MCF-7 cells, which has been demonstrated to be dependent on the function of Kv10.1 channels and modulated by the Akt signalling pathway [22]. TRAM-34 is a novel ER agonist. TRAM-34 was originally developed as a derivative of the commonly used KCa3.1 channel blocker clotrimazole [25] and was selected from a group of prepared clotrimazole derivatives because of its high affinity for KCa3.1 channels (Kd=20 ± 3 nM) and lack of cytochrome P450 inhibition [25]. TRAM-34 differs structurally from clotrimazole by a substituted pyrazole ring in place of the imidazole ring. TRAM-34 has frequently been used to demonstrate a role for KCa3.1 in cellTRAM-34's inhibitory impact (Figure 2). We propose that the anti-proliferative effect of TRAM-34 (and also clotrimazole) is caused by inhibition of KCa3.1 channels, which is consistent with observations in many other cell types [25-31]. In conclusion, K+ channel blockage does not seem to be the cause of the mitogenic action of TRAM-34 at moderate concentrations. The ER-selective modulators ICI and tamoxifen decreased TRAM-34's effect on cell proliferation, which suggests that TRAM-34 may be imitating the effects of E2 to promote cell proliferation (Figure 3). This effect on cell proliferation also appeared to be non-additive with that of E2. TRAM-34 was likewise able to demonstrated two distinct effects of ER activation in MCF-7 cells: boosting PR and reducing ER-a mRNA expression. These effects were also responsive to the ER antagonist ICI. Interestingly, PR mRNA expression was marginally but statistically significantly affected by clotrimazole, indicating that the drug may possibly function as a very weak ER agonist. Clotrimazole did not stimulate proliferation in contrast to TRAM-34, which is consistent with clotrimazole being a considerably weaker ER agonist. Competitive ligand binding experiments demonstrated that TRAM-34 specifically reduced the binding of [3H]-E2 to ER protein, indicating that TRAM-34's action was mediated through ERs. This demonstrates that clotrimazole and TRAM-34 bind to ERs directly around the E2 binding site, indicating that interactions with the ER could be a typical triarylmethane impact. Yet, despite clotrimazole capacity to attach to the ER, our research indicates that only TRAM-34 can stimulate pathways that result in changes in gene expression and an increase in cell proliferation.

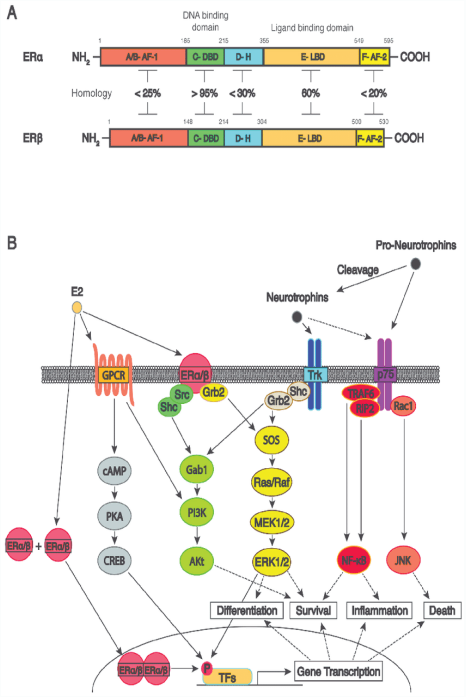

Figure 2: Estrogen receptor structure and function. Homology between ERα and ERβ: amino acid identity (%) in the N-terminal Activation Function 1 region (AF-1), DNA-Binding Domain (DBD), Hinge Region (H), Ligand-Binding Domain (LBD), and C-terminal function 2 domain (AF-2); (B) interaction of neurotrophin receptor and estrogen signaling pathways. Schematic outlining of the neurotrophin receptors and their associated peptides and a simplified diagram describing the main signaling pathways activated by the Trk and p75 receptors. Activation and subsequent phosphorylation of the Trk receptor results in the activation of the MEK/ERK signaling pathway, that leads to phosphorylation of Transcription Factors (TFs) like CREB and promotes cell differentiation and survival; and the PI3K/Akt pathway that promotes cell survival. The p75NTR signals modulate the NF-κB and JNK pathways, which promotes inflammation and cell survival or apoptosis respectively. The classical and non-classical estrogen pathway interacts with neurotrophin system and regulates neuronal survival and ameliorative effects on the BFCN. Dashed arrows indicate various targets.

Conclusion

Research on the function of KCa3.1 in cell physiology frequently makes use of TRAM-34, a high-affinity KCa3.1 channel blocker. It is important to use caution when interpreting results utilizing TRAM-34, as our findings reveal intricate effects that have not been previously documented. TRAM-34 presumably mimics the effects of E2 by interacting directly with the ER in MCF-7 cells. Thus, we propose that TRAM-34 is a novel non-steroidal ER agonist that acts on ER-positive cells without requiring a K+ channel. Our findings provide cautions regarding the in vivo targeting of KCa3.1 channels for therapeutic purposes with TRAM-34 or similar compounds [32,33].

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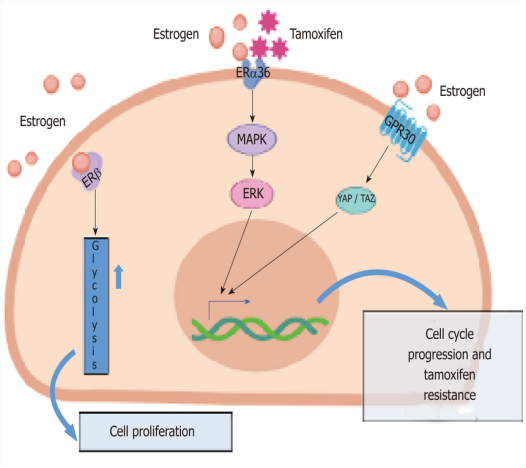

Figure 3: The main pathways activated by different estrogen receptor isoforms in breast cancer stem cells, responsible for cell proliferation and tamoxifen-resistance.
GPER: G-Protein Coupled Receptor; ERα36: Estrogen Receptor Alpha 36; ERβ: Estrogen Receptor Beta; MEK: Mitogen Activated Protein Kinase; ERK: Extracellular Signal regulated Kinase; YAP: Yes-Associated Protein; TAZ: Tafazzin

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