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BMP1 Appears to be Involved in GPER1-mediated Progression and Tamoxifen Resistance of Luminal A Breast Cancer Cells

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Abstract

Background/Aim: Bone morphogenetic protein 1 (BMP1) plays a role in the activation of both transforming growth factor-β (TGFβ) and BMP signaling pathways. We investigated whether BMP1 is involved in G-protein coupled estrogen receptor 1 (GPER1)-regulated progression of luminal A-type breast cancer cells.

Materials and Methods: Publicly available transcriptomic data from MCF7 breast cancer cells treated with the selective GPER1 agonist G1 were analyzed and the results, in particular the altered BMP1 expression, were validated by qPCR. Signs of epithelial-mesenchymal transition (EMT) were visualized by immune cytology. Invasion was quantified by modified Boyden chamber assay. Tamoxifen-resistant sublines of the MCF7 and T47D cell lines were established.

Results: Activation of GPER1 by the agonist G1 increased the expression of BMP1 in MCF7 and T47D luminal A breast cancer cells. In addition, EMT and invasion was enhanced after GPER1 activation. This effect could be prevented in part by the BMP1 inhibitor UK383367. Tamoxifen-resistant MCF7-TR and T47D-TR cells exhibited higher BMP1 expression, signs of EMT and enhanced invasiveness compared to their tamoxifen-sensitive wild type. Blocking GPER1 in MCF7-TR and T47D-TR cells using the antagonist G36 led to reduction in BMP1 expression, a slight decrease in EMT, reduced cell invasion, and increased sensitivity to tamoxifen.

Conclusion: BMP1 appears to be involved in GPER1-mediated progression of luminal A breast cancer cells. In addition, BMP1 may play a role in tamoxifen-resistance.

Keywords: Bone morphogenetic protein 1, BMP1, G-protein coupled estrogen receptor 1, GPER1, luminal A breast cancer, tamoxifen-resistance.

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Introduction

Breast cancer remains one of the most prevalent and heterogeneous malignancies affecting women worldwide (1). Among its various subtypes, luminal A breast cancer is characterized by estrogen receptor (ER) positivity, progesterone receptor (PR) positivity, and low levels of the human epidermal growth factor receptor 2 (HER2) (2). This subtype is generally associated with a favorable prognosis due to its responsiveness to endocrine therapies such as tamoxifen and aromatase inhibitors (3). However, despite advances in treatment, challenges such as therapeutic resistance and disease recurrence persist, necessitating a deeper understanding of the molecular mechanisms underlying luminal A breast cancer progression (4, 5).

The G protein-coupled estrogen receptor (GPER1), a membrane-bound receptor mediating rapid estrogen signaling, has been implicated in diverse physiological and pathological processes, including cell proliferation, inflammation, and metabolic regulation (6, 7). GPER1 is expressed by many hormone-sensitive tumors and rapidly activates signaling cascades mediated by estrogen, making it a potential target for treatment (8). GPER1 has been detected in tissues including the testis, ovaries, breast, endometrium, and lung (9). It is likely that GPER1 modulates carcinogenesis (10). The tissue type seems to determine the effects of the receptor (9). In cervical carcinoma, tumor suppressive (10-14) and oncogenic effects (15-17) are under discussion. Both the histological subtype and the expression levels of GPER1 appear to be decisive (18, 19).

In breast cancer GPER1 is often described as an oncogene (20-24) but Han *et al.* (25) observed a tumor suppressive effect. Inhibition of GPER1 prevents growth stimulation of triple-negative breast cancer (TNBC) cells by 17β-estradiol (26). In luminal breast cancer, GPER1 is associated with the development of tamoxifen resistance (27).

Bone morphogenetic protein 1 (BMP1), a metallo-protease of the tolloid family, primarily regulates extracellular matrix (ECM) remodeling by processing procollagens and other ECM components, while also modulating BMP/TGF-β signaling pathways (28). The involvement of BMP1 in cancer is complex and context-dependent. On one hand, BMP1-mediated ECM remodeling can promote tumor progression by facilitating cancer cell invasion and metastasis. For example, in TNBC, BMP1 has been shown to enhance tumor aggressiveness by degrading basement membrane components (*e.g.*, collagen IV) and promoting angiogenesis (29). On the other hand, BMP1 may also exert tumor-suppressive effects by maintaining ECM integrity and preventing aberrant cell proliferation, as observed in colorectal cancer models where BMP1 loss correlated with increased stromal disruption and tumor growth (30). In luminal A breast cancer, the role of BMP1 is less clear. Given the relatively low aggressiveness of this subtype, it is plausible that BMP1 may contribute to the maintenance of a structured tumor microenvironment, thereby limiting invasive potential. Whether this is the case needs to be verified. However, emerging evidence suggests that BMP1 may also play a role in therapeutic resistance. For instance, BMP1-mediated activation of TGF-β signaling has been linked to the epithelial-mesenchymal transition (EMT), a process associated with therapy resistance in breast cancer (31, 32).

Although no direct molecular interaction between GPER1 and BMP1 has been reported to date, their functional overlap in tissue homeostasis suggests potential crosstalk. For instance, GPER1 activation influences ECM composition –*e.g.*, by upregulating collagen deposition in fibrosis (33)– a process BMP1 directly controls via cleavage of procollagen (34). Additionally, estrogen (acting through GPER1) is known to modulate BMP/TGF-β signaling (35), hinting at an indirect regulatory axis. Both proteins are also implicated in cancer progression: GPER1 promotes tumorigenesis in hormone-sensitive cancers (36), while BMP1 facilitates metastasis via ECM remodeling (29). Further research is needed to elucidate whether these pathways converge mechanistically, particularly in contexts like tumor microenvironment dysregulation or tumor progression.

Materials and Methods

Analysis of next generation sequencing (NGS) data. Publicly available transcriptomic data from MCF7 breast cancer cells treated with the selective GPER1 agonist G1 were analyzed (37). Total RNA was isolated from MCF7 cells treated with 100 nM G1 (*n*=3), 1 μM G1 (*n*=3), or vehicle (0.1% ethanol, *n*=3) for 48 h. Details of library preparation, quality checking and trimming, read mapping against the human genome, and quantification of mapped reads are described in (37). Raw sequence and processed data are published in Gene Expression Omnibus (GEO accession number GSE188706). Raw fastq files were processed using Galaxy software (v20.01). Following quality assessment with FastQC (v0.6.5), sequencing reads were trimmed (FASTQ Trimmer v1.1.5) and aligned to the hg38 reference genome using RNAstar (v2.7.8a). Genomic feature assignment was performed with FeatureCounts (v2.0.8), and differential gene expression analysis was conducted using DESeq2 (v2.0.1). For functional enrichment analysis, Gene Set Enrichment Analysis (GSEA, v4.3.3) and the Enrichr web tool were employed. GSEA was executed on a count matrix filtered for genes with expression above background levels (basemean >15 normalized counts), using the "Hallmarks of Cancer" gene set database (H.all.v7.0). A ranking list of log2FC values and adjusted p-values of the expressed genes were made available by GSEA analysis.

Cell culture. The human breast cancer cell lines MCF7 and T47D were acquired from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were cultured in Minimum Essential Medium (MEM; Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal calf serum (FCS; Pan Biotech, Aidenbach, Germany), 0.1% transferrin (Merck, Darmstadt, Germany), 1% penicillin/streptomycin (Thermo Fisher Scientific), and 26 IU insulin (Sanofi, Paris, France), and maintained at 37˚C in a 5% CO_2 humidified atmosphere. To ensure cell line integrity, purchased stocks were expanded, aliquoted, and stored in liquid nitrogen. Fresh frozen stocks were thawed every six months, and routine mycoplasma testing was performed using a PCR-based kit (Mycoplasma Test Kit I/C, Vazyme, Düsseldorf, Germany).

Drugs. GPER1 agonist G1 (#TGM-T40517), GPER1 antagonist G36 (#TGM-T22794), and BMP1 inhibitor UK383367 (#TGM-T6064) were purchased from Biomol (Hamburg, Germany). 4-hydroxytamoxifen (4-OHT), the active metabolic form of tamoxifen, was acquired by Merck. G1, G36, 4-OHT, and UK383367 were dissolved in ethanol. Each control was treated with 0.03 v/v % ethanol.

Development of tamoxifen-resistant cells. Tamoxifen-resistant sublines MCF7-TR and T47D-TR were developed as described previously (38). A 4-OHT medium concentration of 125 nM was used. The cells were cultured at 37˚C in a humidified atmosphere of 5% CO2 in air as previously described (38).

Phalloidin staining. Cells grown on round coverslips were washed with Dulbecco's phosphate buffered saline (DPBS; PAN-Biotech), fixed in 4% paraformaldehyde (PFA; Merck) for 10 min at room temperature (RT), and permeabilized with 1% Triton X-100 (Merck) in PBS for 5 min. After blocking with 2% bovine serum albumin (BSA; Carl Roth, Karlsruhe, Germany) in PBS (30 min), cells were incubated with Phalloidin CruzFluor™ 555 (1:4,000; Santa Cruz Biotechnology, Heidelberg, Germany) and DAPI (1:1,000; Novus Biologicals, Centennial, CO, USA) for 30 min (protected from light). Coverslips were mounted with Mowiol (Merck) and imaged using an Olympus IX83 microscope with cellSens Dimension software v. 4.4 (Olympus Life Science Solutions, Tokyo, Japan). Staining was quantified in ImageJ v. 2.14.0 (NIH, Bethesda, MD, USA).

Immunofluorescence staining. Cells on coverslips were treated as above. Fixed samples were incubated with a primary anti-vimentin antibody (1:250; Abcam, Cambridge, UK) at 4˚C for 1 h, followed by a secondary Alexa Fluor 488-conjugated antibody (Thermo Fisher Scientific) and DAPI (1 μg/ml; Novus Biologicals) for

30 min (RT, dark). After washing, slides were mounted with fluorescence mounting medium (Agilent Dako, Santa Clara, CA, USA) and imaged using an Olympus IX83 microscope with cellSens Dimension software v. 4.4 (Olympus Life Science Solutions). Staining was quantified in ImageJ v. 2.14.0 (NIH).

Real-time quantitative PCR analysis. Total RNA was extracted using a RNeasy Mini Kit (Qiagen, Hilden, Germany), and 2 μg of RNA was reverse-transcribed with a High-Capacity cDNA Kit (Qiagen). qPCR was performed with SYBR Green Master Mix (Qiagen) using primers for BMP1 (F: 5'-TAC ACC TAT GAC CTG GCG GA -3'; R: 5'- ACC TCT CCA TCT CCC ACA GG -3') and GAPDH (F: 5'-GAA GGT CGG AGT CAA CGG AT-3'; R: 5'-TGG AAT TTG CCA TGG GTG GA-3'; Merck). Cycling conditions were the following: 95˚C (2 min), followed by 40 cycles of 95˚C (5 s) and 60˚C (15 s).

Viability. Cells seeded in 96-well plates (Corning Life Sciences, Amsterdam, the Netherlands) in phenol-red-free Dulbecco's Minimum Essential Medium (DMEM, Thermo Fisher) were treated for 24 h. After 72 h, 20 μl Resazurin (Thermo Fisher) was added per well. Fluorescence (570/630 nm) was measured after 10 h using a BioTek microplate reader and the GEN5 software v. 3.17.16 (BioTek Instruments, Winooski, VT, USA). Data analysis was performed in Excel v. 1808 (Microsoft, Redmond, WA, USA).

Transwell co-culture invasion assay. Using the co-culture transwell assay, as described earlier (39), 1×10^4 breast cancer cells in phenol-red-free DMEM+10% cs-FCS were seeded into Matrigel® (BD Bioscience, Bedford, MA, USA) or gelatin (1 mg/m in PBS, Sigma) coated inserts (8 μm pores; Merck Millipore, Cork, Ireland). 2.5×10^4 osteosarcoma cells (with/without 10% cs-FCS) were plated in the lower chamber to stimulate invasion of the breast cancer cells in the upper chamber (39). After 24 h, cells were co-cultured for 96 h. Invaded cells were hematoxylin-stained, and four random fields per insert were counted.

Statistical analysis. All experiments were performed in ≥3 biological and ≥2 technical replicates. Data are presented as mean±standard error of the mean (SEM). Statistical significance ($p<0.05$) was determined using one-way ANOVA (followed by Tukey's test) or unpaired two-tailed *t*-tests (assuming equal variance; Prism v. 8.4.3, GraphPad Software Inc., San Diego, CA, USA). Gene set enrichment analysis (GSEA) was performed for NGS data visualization and heatmaps were generated in Prism v. 8.4.3 (GraphPad Software Inc.).

Results

Expression of "Hallmarks of Cancer" genes in MCF7 cells treated with the GPER1 agonist G1. To investigate molecular differences in gene expression associated with GPER1 activation in luminal A breast cancer cells, publicly available transcriptomic raw data from MCF7 breast cancer cells treated with the selective GPER1 agonist G1 (37) were analyzed. One of the most upregulated pathways from Hallmark Gene Set datasets was the epithelial-mesenchymal transition (EMT) pathway (Figure 1A). The signaling pathway of EMT showed differences in the GSEA profile of MCF7 cells with G1 treatment. The genes of this gene set were upregulated in G1-treated cells and downregulated in the control cells (Figure 1B). The genes shown in the heatmap also showed stronger upregulation in G1-treated MCF7 cells (Figure 1C). One of the most upregulated genes from mRNA sequencing responsible for EMT progression was the bone morphogenetic protein 1 (BMP1) (Figure 1C).

Effects of G1 treatment on EMT, BMP1 expression, and invasion of MCF7 and T47D cells. Treatment of luminal A breast cancer cells with the GPER1 agonist G1 resulted in significantly increased signs of EMT, as evidenced by increased immunocytologic detection of vimentin expression ($p<0.01$ *vs.* MCF7 control, $n=3$; Figure 2A–C) and significant increased phalloidin staining ($p<0.01$ *vs.* MCF7 control, v3; Figure 2D–F). Relative expression of BMP1 was significantly increased in both MCF7 and T47D

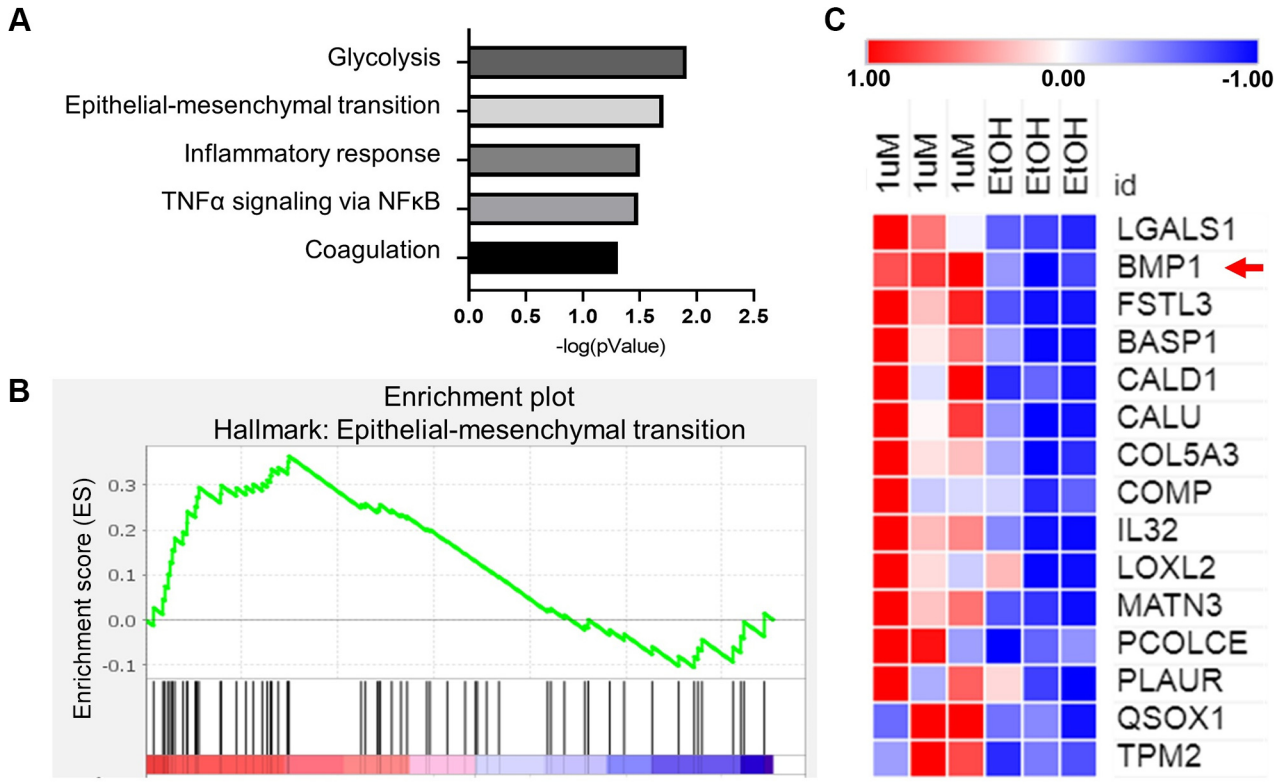

Figure 1. *Gene expression analysis of MCF7 cells treated with or without G-protein coupled estrogen receptor 1 (GPER1) agonist G1. A) Most upregulated pathways from Hallmark Gene Set datasets. B) Illustration of enrichment plots from Gene Set Enrichment Analysis (GSEA) of RNA sequencing in relation to epithelial-mesenchymal transition (EMT)-specific genes. C) Representation of genes from RNA sequencing that are responsible for EMT progression.*

cell lines after treatment with G1 (p<0.01 and p<0.05 *vs.* control, for MCF7 and T47D, respectively, n=3 for both; Figure 2E). Invasion was also significantly increased in both cell lines by G1 treatment (p<0.001 and p<0.0001 *vs.* control, for MCF7 and T47D, respectively, n=6 for both; Figure 2F). This effect could be significantly reduced by BMP1 inhibitor UK383367 (p<0.05 *vs.* G1 treatment, for MCF7 and T47D, n=6 for both; Figure 2F).

EMT, BMP1 expression, and invasion of tamoxifen-resistant MCF7-TR and T47D-TR cells. First, sublines of ERα-positive breast cancer cell lines MCF7 (Figure 3A) and T47D (Figure 3B) with secondary resistance against 4-hydroxy-tamoxifen (4-OHT) were generated according to Günthert *et al.* (38) using a medium concentration of 4-OHT of 125 nM. Treatment of parental MCF7 cells with 5 μM 4-OHT resulted in a significant reduction of viability (p<0.001 *vs.* MCF7 control, n=5; Figure 3A), while viability of tamoxifen-resistant MCF7-TR cells remained unchanged (n=5; Figure 3A). Viability of parental T47D cells treated with 5 μM 4-OHT was significantly reduced (p<0.01 *vs.* T47D control, n=5; Figure 3B). Tamoxifen-resistant T47D-TR cells treated with 5 μM 4-OHT showed no changes in viability (n=5; Figure 3B). In the next step, signs of EMT in MCF7 wild-type cells (Figure 4A, E) and the tamoxifen-resistant subline MCF7-TR (Figure 4B, F) were compared. The tamoxifen-resistant cells showed significantly increased vimentin detection (p<0.001 *vs.* MCF7 wild-type, n=3; Figure 4B, D) and phalloidin staining (p<0.01 *vs.* MCF7 wild-type, n=3; Figure 4F, H). In addition, BMP1 expression (Figure 5A) and invasion (Figure 5C) of the tamoxifen-resistant sublines MCF7-TR and T47D-TR were

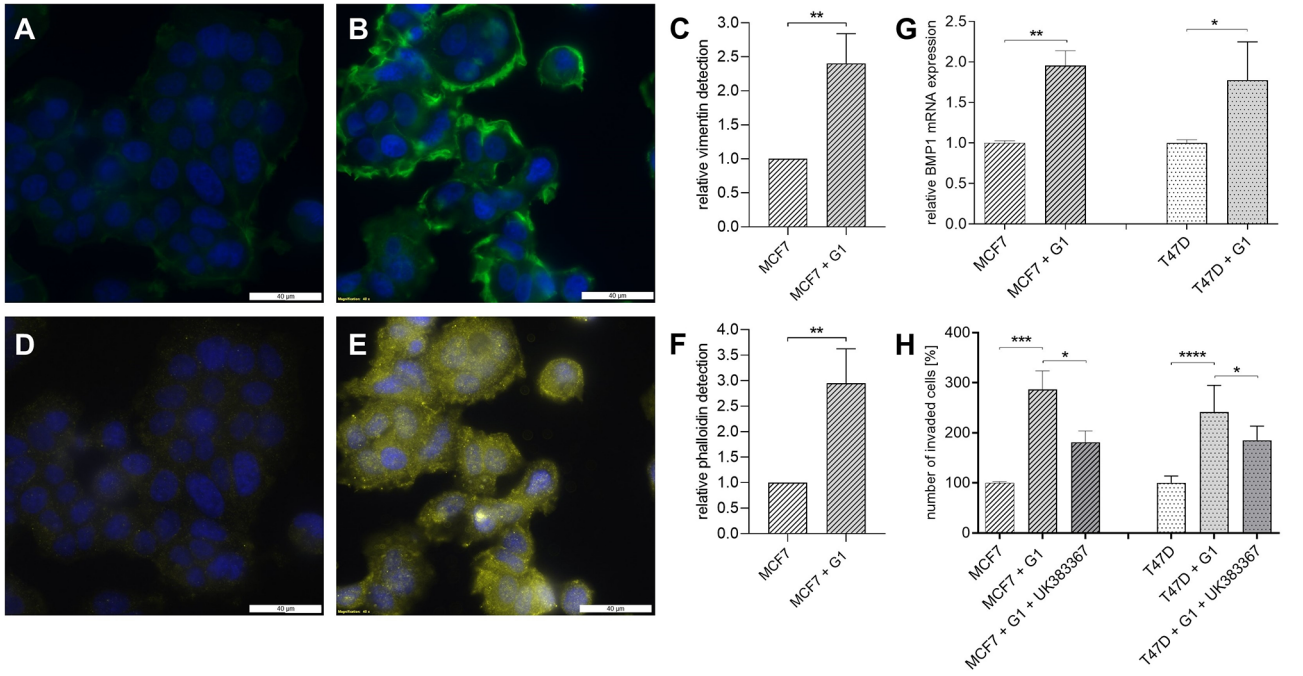

Figure 2. *Effects of G1 treatment on epithelial-mesenchymal transition (EMT), expression of bone morphogenetic protein 1 (BMP1), and invasion of MCF7 and T47D cells. Visualization (A, B, D, E) and quantification (C, F) of EMT progression after G-protein coupled estrogen receptor 1 (GPER1) stimulation with the GPER1 agonist G1 in MCF7 cells using immunocytological fluorescence staining. (A, D) untreated control; (B, E) GPER1 activation using 1 μM G1. Cell nuclei are shown in blue (A, B, D, E), vimentin in green (A, B), phalloidin in yellow (D, E). Effects of G1 treatment on BMP1 expression of MCF7 and T47D cells (G). Effects of G1 treatment without or with BMP1 inhibitor UK383367 on invasion of MCF7 and T47D cells (H). Mean±standard error of the mean (SEM), unpaired t-test (C, F, G), one-way ANOVA followed by Tukey's test (H), n=3 (C, F, G), n=6 (H), $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, $^{****}p<0.0001$.*

compared with the parental MCF7 and T47D cell lines. BMP1 expression of tamoxifen-resistant MCF7-TR and T47D-TR cells was significantly higher as compared with the tamoxifen-sensitive MCF7 and T47D wild type cells (MCF7-TR: $p<0.001$ *vs.* MCF7, $n=6$; T47D-TR: $p<0.05$ *vs.* T47D, $n=6$; Figure 5A). Furthermore, invasion of tamoxifen-resistant MCF7-TR and T47D-TR cells was significantly increased as compared with the tamoxifen-sensitive MCF7 and T47D wild type cells (MCF7-TR: $p<0.001$ *vs.* MCF7, $n=6$; T47D-TR: $p<0.01$ *vs.* T47D, $n=6$; Figure 5C).

Effects of GPER1 antagonist G36 treatment on EMT, BMP1 expression, and invasion of tamoxifen-resistant MCF7-TR and T47D-TR cells. Treatment of tamoxifen-resistant breast cancer cells with GPER1 antagonist G36 resulted in significantly decreased signs of EMT, as evidenced by significantly decreased immunocytologic detection of vimentin expression ($p<0.001$ *vs.* MCF7-TR, $n=3$; Figure 4C, D) and significantly decreased phalloidin staining ($p<0.01$ *vs.* MCF7-TR, $n=3$; Figure 4G, H). Expression of BMP1 was significantly decreased in both MCF7-TR and T47D-TR cell lines after treatment with G36 ($p<0.01$ and $p<0.05$ *vs.* control, for MCF7-TR and T47D-TR, respectively, $n=3$ for both; Figure 5B). Invasion was also significantly decreased in both cell lines by G36 treatment ($p<0.05$ and $p<0.01$ *vs.* control, for MCF7-TR and T47D-TR, respectively, $n=6$ for both; Figure 5D).

Effects of GPER1 antagonist G36 treatment on tamoxifen resistance of MCF7-TR and T47D-TR cells. Finally, it was examined whether blocking of GPER1 using the GPER1 antagonist G36 has an influence on the resistance to tamoxifen of MCF7-TR and T47D-TR cell lines. As shown

above, tamoxifen had no effect on the viability of tamoxifen-resistant MCF7-TR and T47D-TR cells (Figure 3 and Figure 6). Treatment with the GPER1 antagonist G36 alone showed a slight non-significant decrease in the viability of MCF7-TR cells (Figure 6A). G36 treatment had no inhibitory effect on viability of T47D-TR cells (Figure 6B). However, the efficacy of tamoxifen on viability of MCF7-TR and T47D-TR cells was restored by treatment with the GPER1 antagonist G36 (MCF7-TR: $p<0.01$ *vs.* control, $p<0.01$ *vs.* 4-OHT treatment, $p<0.05$ *vs.* G36 treatment, $n=3$, Figure 6A; T47D-TR: $p<0.001$ *vs.* control, $p<0.01$ *vs.* 4-OHT treatment, $p<0.01$ *vs.* G36 treatment, $n=4$, Figure 6B).

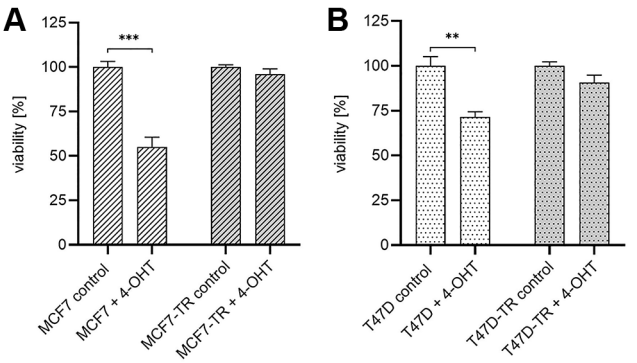

Figure 3. *Proof of successful generation of tamoxifen-resistant MCF7-TR and T47D-TR cells. Viability of MCF7 wild-type cells and tamoxifen-resistant MCF7-TR cells (A), and T47D wild-type cells and tamoxifen-resistant T47D-TR cells (B), after treatment without or with 4-hydroxytamoxifen (4-OHT). The control shows viability in culture medium with 0.05% ethanol. Mean±standard error of the mean (SEM), unpaired t-test, n=5. $^{**}p<0.01$, $^{***}p<0.001$.*

Discussion

Activation of G-protein coupled estrogen receptor 1 (GPER1) via its agonist G1 significantly increased the expression of bone morphogenetic protein 1 (BMP1) in MCF7 and T47D luminal A breast cancer cells. In addition, epithelial-mesenchymal transition (EMT) and invasion were enhanced after GPER1 activation. These effects could be partially prevented by the BMP1 inhibitor UK383367. MCF7-TR and T47D-TR cells that have acquired resistance to tamoxifen show higher BMP1 expression, signs of EMT and increased invasiveness compared to the respective tamoxifen-sensitive wild types. Blocking GPER1 in MCF7-TR and T47D-TR cells with the GPER1 antagonist G36 led to a reduction in BMP1 expression, a slight decrease in EMT, reduced cell invasion and increased sensitivity to tamoxifen.

Our data show that BMP1 plays a pivotal role in GPER1-dependent progression of luminal A breast cancer cells. This is consistent with the established function of BMP1 as an activator of both transforming growth factor-β (TGFβ) and BMP signaling pathways (28). The observation that GPER1 activation by agonist G1 increases BMP1 expression in MCF7 and T47D cells expands the current understanding of estrogen-independent signaling mechanisms in hormone receptor-positive tumors. In particular, the resulting increase in EMT and invasion underscores the importance of BMP1- extracellular matrix (ECM) remodeling for tumor progression. The fact that this effect could be partially reversed by BMP1 inhibitor UK383367 suggests a direct link - a finding supported by studies describing BMP1 as a key protease for TGF-β release from the ECM (28, 40).

Particularly relevant is the discovery that tamoxifen-resistant cells (MCF7-TR/T47D-TR) have elevated BMP1 levels. This suggests a previously undescribed mechanism of resistance development: chronic tamoxifen exposure could perpetuate BMP1-dependent TGF-β activation via GPER1 upregulation, which contributes to EMT and therapy resistance. This is also supported by the fact that GPER1 blockade by G36 in resistant cells not only decreased BMP1 expression but also restored tamoxifen sensitivity. This finding complements current models of stroma-mediated resistance (41) by identifying BMP1 as a mechanistic link between GPER1 signaling and ECM remodeling.

Clinically, this raises the question of whether BMP1 could serve as a predictive biomarker for tamoxifen resistance. However, the specificity of this effect needs to be further investigated - especially in light of the fact that BMP1 shows tumor-suppressive effects in other contexts (*e.g.*, colorectal carcinomas) (30). In addition, further

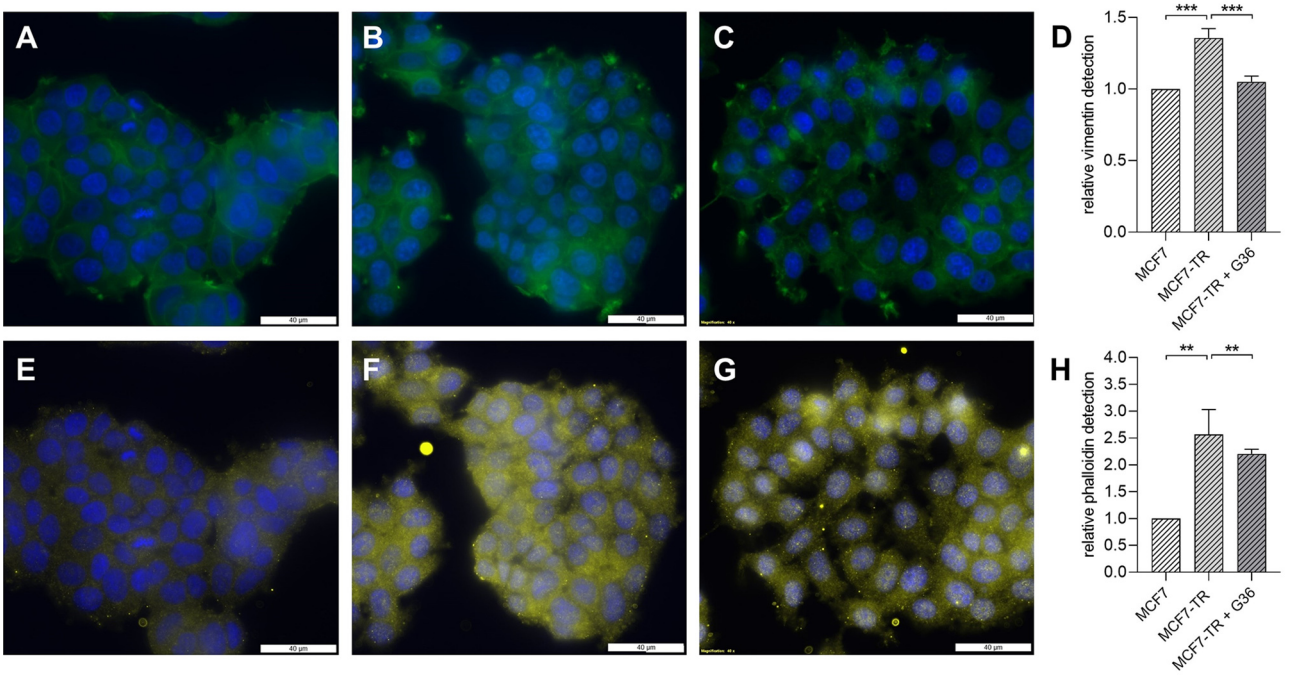

Figure 4. *Epithelial-mesenchymal transition (EMT) in tamoxifen-sensitive MCF7 cells, tamoxifen-resistant MCF7-TR cells and MCF7-TR cells treated with G-protein coupled estrogen receptor 1 (GPER1) antagonist G36. Visualization (A-C, E-G) and quantification (D, H) of EMT in MCF7 wild-type cells (A, E), tamoxifen-resistant MCF7-TR cells (B, F), and tamoxifen-resistant MCF7-TR cells after GPER1 blockade with GPER1 antagonist G36 (C, G) using immunocytological fluorescence staining. (A, E) untreated MCF7; (B, F) untreated MCF7-TR; (C, G) MCF7-TR treated with 10 μM GPER1 antagonist G36. Cell nuclei are shown in blue (A-C, E-G), vimentin in green (A-C), phalloidin in yellow (E-G). (D, H) Mean±standard error of the mean (SEM), one-way ANOVA followed by Tukey's test, n=3, $^{**}p<0.01$, $^{***}p<0.001$.*

factors influence tamoxifen resistance. For example, 17β-estradiol suppresses expression of Noggin, which when overexpressed enhances proliferation and promotes resistance to tamoxifen and other drugs (42). It cannot be ruled out that GPER1 intervenes here, possibly via BMP1. Numerous other factors interact with BMP1; for example, fibronectin can bind BMP1 and regulate its function (43). Takeyama *et al.* found that blood levels of sialic acid fibronectin, an autocrine fibronectin which is secreted by breast cancer cells, are associated with drug resistance, EMT and clinical prognosis (44). Future studies should therefore characterize BMP1 substrates in GPER1-activated cells using proteomics, analyze the influence of stromal *vs.* tumoral BMP1 expression in patient profiles and preclinically test the efficacy of BMP1-inhibiting substances in combination with endocrine therapies.

Conclusion

In summary, activation of GPER1 in luminal A breast cancer cells by the agonist G1 increased expression of BMP1. In addition, EMT and invasion were enhanced. This could be partially prevented by the BMP1 inhibitor UK383367 (Figure 7). Tamoxifen-resistant breast cancer cells showed higher BMP1 expression, enhanced EMT and increased invasiveness compared to tamoxifen-sensitive wild type breast cancer cells (Figure 7). Blocking GPER1 in tamoxifen-resistant breast cancer cells with GPER1 antagonist G36 led to a reduction in BMP1 expression, a decrease in EMT, reduced invasion and increased sensitivity to tamoxifen (Figure 7). These data establish BMP1 as a promising therapeutic target in luminal A breast cancers with GPER1 overexpression or tamoxifen resistance. The results underline the importance of

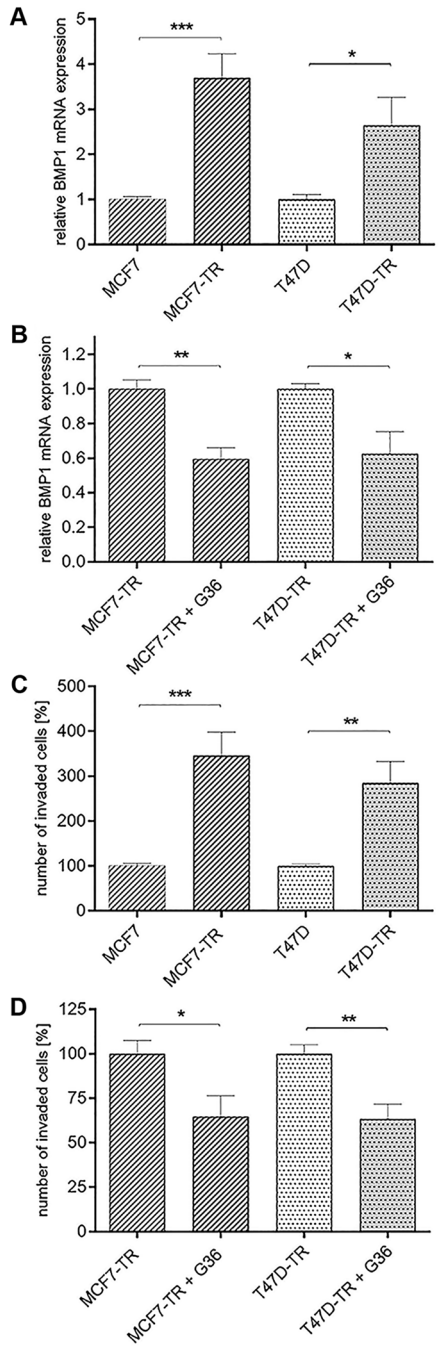

Figure 5. *Bone morphogenetic protein 1 (BMP1) expression and invasion of breast cancer cells treated with or without the G-protein coupled estrogen receptor 1 (GPER1) antagonist G36. BMP1 expression (A) and invasion (C) in tamoxifen-sensitive MCF7 and T47D wild-type cells and tamoxifen-resistant MCF7-TR and T47D-TR cells. Effect of GPER1 antagonist G36 on BMP1 expression (B) and invasion (D) of MCF7-TR and T47D-TR cells. Mean±standard error of the mean (SEM), unpaired t-test, n=3 (B), n=6 (A, C, D), $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.*

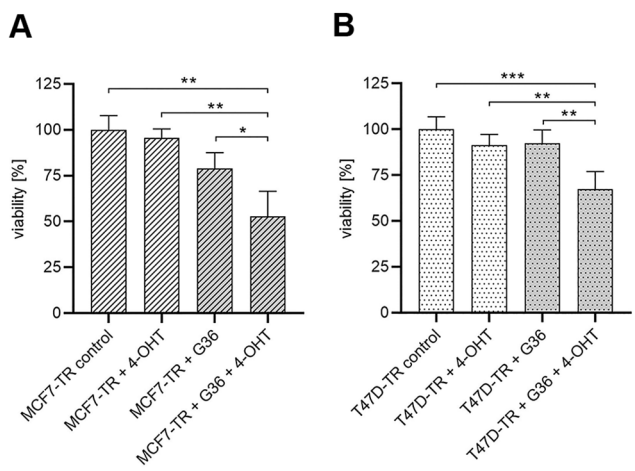

Figure 6. *Effects of the G-protein-coupled estrogen receptor 1 (GPER1) antagonist G36 on tamoxifen efficacy in tamoxifen-resistant breast cancer cells. Viability of tamoxifen-resistant MCF7-TR (A) and T47D-TR (B) cells after treatment without or with 4-OHT and/or GPER1 antagonist G36. The control shows viability in culture medium with 0.05% ethanol. Mean±standard error of the mean (SEM), one-way ANOVA followed by Tukey's test, n=3 (A), n=4 (B), $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.*

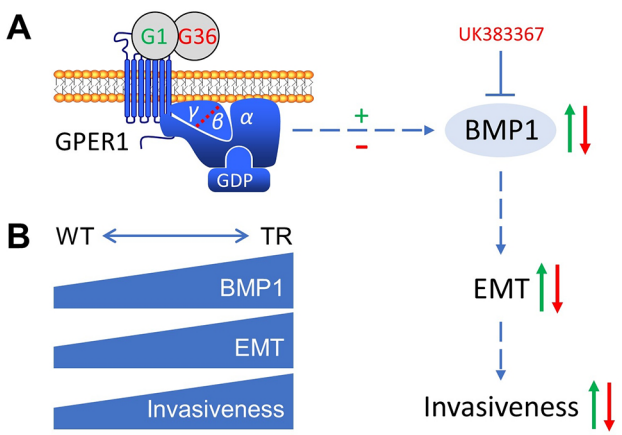

Figure 7. *Mechanisms of action of the G-protein coupled estrogen receptor 1 (GPER1) - bone morphogenetic protein 1 (BMP1) axis in luminal A breast cancer cells. A) Activation of G-protein coupled estrogen receptor 1 (GPER1) by the agonist G1 increases expression of bone morphogenetic protein 1 (BMP1), epithelial-mesenchymal transition (EMT) and invasion of luminal A breast cancer cells. BMP1 inhibition by UK383367 prevents in part increased EMT and invasion. GPER1 antagonist G36 reduces BMP1 expression, EMT and invasion. B) Tamoxifen-resistant (TR) breast cancer cells show increased BMP1 expression, EMT and invasion, compared to tamoxifen-sensitive wild type (WT) breast cancer cells.*

microenvironment interaction for tumor progression and provide new targets to overcome therapy-limiting resistance mechanisms.

Conflicts of Interest

The Authors declare no conflicts of interest.

Authors' Contributions

Conceptualization, C.G.; investigation, K.W. and J.J.; writing – original draft preparation, C.G.; writing – review and editing, J.G.; project administration, C.G. All Authors have read and agreed to the published version of the manuscript.

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References

1 Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F: Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 71(3): 209-249, 2021. DOI: 10.3322/caac.21660

2 Goldhirsch A, Winer EP, Coates AS, Gelber RD, Piccart-Gebhart M, Thürlimann B, Senn HJ, Panel members: Personalizing the treatment of women with early breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013. Ann Oncol 24(9): 2206-2223, 2013. DOI: 10.1093/annonc/mdt303

3 Early Breast Cancer Trialists' Collaborative Group (EBCTCG): Aromatase inhibitors versus tamoxifen in early breast cancer: Patient-level meta-analysis of the randomised trials. Lancet 386(10001): 1341-1352, 2015. DOI: 10.1016/S0140-6736(15)61074-1

4 Hanker AB, Sudhan DR, Arteaga CL: Overcoming endocrine resistance in breast cancer. Cancer Cell 37(4): 496-513, 2020. DOI: 10.1016/j.ccell.2020.03.009

5 Jeselsohn R, Buchwalter G, De Angelis C, Brown M, Schiff R: ESR1 mutations—a mechanism for acquired endocrine resistance in breast cancer. Nat Rev Clin Oncol 12(10): 573-583, 2015. DOI: 10.1038/nrclinonc.2015.117

6 Prossnitz ER, Barton M: The G-protein-coupled estrogen receptor GPER in health and disease. Nat Rev Endocrinol 7(12): 715-726, 2011. DOI: 10.1038/nrendo.2011.122

7 Prossnitz ER, Barton M: The G protein-coupled oestrogen receptor GPER in health and disease: an update. Nat Rev Endocrinol 19(7): 407-424, 2023. DOI: 10.1038/s41574-023-00822-7

8 Bai LY, Weng JR, Hu JL, Wang D, Sargeant AM, Chiu CF: G15, a GPR30 antagonist, induces apoptosis and autophagy in human oral squamous carcinoma cells. Chemicobiol Inter 206(2): 375-384, 2013. DOI: 10.1016/j.cbi.2013.10.014

9 Jacenik D, Cygankiewicz AI, Krajewska WM: The G protein-coupled estrogen receptor as a modulator of neoplastic transformation. Mol Cell Endocrinol 429: 10-18, 2016. DOI: 10.1016/j.mce.2016.04.011

10 Hernández-Silva CD, Villegas-Pineda JC, Pereira-Suárez AL: Expression and role of the G protein-coupled estrogen receptor (GPR30/GPER) in the development and immune response in female reproductive cancers. Front Endocrinol (Lausanne) 11: 544, 2020. DOI: 10.3389/fendo.2020.00544

11 Friese K, Kost B, Vattai A, Marmé F, Kuhn C, Mahner S, Dannecker C, Jeschke U, Heublein S: The G protein-coupled estrogen receptor (GPER/GPR30) may serve as a prognostic marker in early-stage cervical cancer. J Cancer Res Clin Oncol 144(1): 13-19, 2018. DOI: 10.1007/s00432-017-2510-7

12 Ramírez-López IG, Ramírez de Arellano A, Jave-Suárez LF, Hernández-Silva CD, García-Chagollan M, Hernández-Bello J, Lopez-Pulido EI, Macias-Barragan J, Montoya-Buelna M, Muñoz-Valle JF, Pereira-Suárez AL: Interaction between 17β-estradiol, prolactin and human papillomavirus induce E6/E7 transcript and modulate the expression and localization of hormonal receptors. Cancer Cell Int 19: 227, 2019. DOI: 10.1186/s12935-019-0935-6

13 Ruckriegl S, Loris J, Wert K, Bauerschmitz G, Gallwas J, Gründker C: Knockdown of G protein-coupled estrogen receptor 1 (GPER1) enhances tumor-supportive properties in cervical carcinoma cells. Cancer Genomics Proteomics 20(3): 281-297, 2023. DOI: 10.21873/cgp.20381

14 Rörig L, Ruckriegl S, Gallwas J, Gründker C: G Protein-coupled estrogen receptor 1 (GPER1) regulates expression of SERPINE1/PAI-1 and inhibits tumorigenic potential of cervical

squamous cell carcinoma cells in vitro. Cancer Genomics Proteomics 22(1): 13-23, 2025. DOI: 10.21873/cgp.20482
15 Akimoto T, Takasawa A, Takasawa K, Aoyama T, Murata M, Osanai M, Saito T, Sawada N: Estrogen/GPR30 signaling contributes to the malignant potentials of ER-negative cervical adenocarcinoma via regulation of Claudin-1 expression. Neoplasia 20(10): 1083-1093, 2018. DOI: 10.1016/j.neo.2018.08.010
16 Ino Y, Akimoto T, Takasawa A, Takasawa K, Aoyama T, Ueda A, Ota M, Magara K, Tagami Y, Murata M, Hasegawa T, Saito T, Sawada N, Osanai M: Elevated expression of G protein-coupled receptor 30 (GPR30) is associated with poor prognosis in patients with uterine cervical adenocarcinoma. Histol Histopathol 35(4): 351-359, 2020. DOI: 10.14670/hh-18-157
17 Zhu CX, Xiong W, Wang ML, Yang J, Shi HJ, Chen HQ, Niu G: Nuclear G protein-coupled oestrogen receptor (GPR30) predicts poor survival in patients with ovarian cancer. J Int Med Res 46(2): 723-731, 2018. DOI: 10.1177/0300060517717625
18 Hambach L, Gallwas J, Gründker C: G-Protein-coupled estrogen receptor 1 (GPER1) overexpression affects aggressiveness of cervical carcinoma cells depending on histological entity. Cancer Genomics Proteomics 22(3): 397-414, 2025. DOI: 10.21873/cgp.20509
19 Hambach L, Gallwas J, Gründker C: Expression of "Hallmarks of Cancer" genes in cervical carcinoma is differentially affected by GPER1 overexpression depending on histologic entity. Cancer Genomics Proteomics 22(3): 415-433, 2025. DOI: 10.21873/cgp.20510
20 Girgert R, Emons G, Gründker C: Inhibition of growth hormone receptor by Somavert reduces expression of GPER and prevents growth stimulation of triple-negative breast cancer by 17β-estradiol. Oncol Lett 15(6): 9559-9566, 2018. DOI: 10.3892/ol.2018.8521
21 Liu Y, Du FY, Chen W, Fu PF, Yao MY, Zheng SS: G15 sensitizes epithelial breast cancer cells to doxorubicin by preventing epithelial-mesenchymal transition through inhibition of GPR30. Am J Transl Res 7(5): 967-975, 2015.
22 Molina L, Bustamante F, Ortloff A, Ramos I, Ehrenfeld P, Figueroa CD: Continuous exposure of breast cancer cells to tamoxifen upregulates GPER-1 and increases cell proliferation. Front Endocrinol (Lausanne) 11: 563165, 2020. DOI: 10.3389/fendo.2020.563165
23 Schmitz V, Bauerschmitz G, Gallwas J, GrÜndker C: Suppression of G protein-coupled estrogen receptor 1 (GPER1) enhances the anti-invasive efficacy of selective ERβ agonists. Anticancer Res 42(11): 5187-5194, 2022. DOI: 10.21873/anticanres.16025
24 Yang SY, Lee JW, Kim WS, Jung KL, Lee SJ, Lee JH, Bae DS, Kim BG: Adenoid cystic carcinoma of the Bartholin's gland: Report of two cases and review of the literature. Gynecol Oncol 100(2): 422-425, 2006. DOI: 10.1016/j.ygyno.2005.08.030
25 Han N, Heublein S, Jeschke U, Kuhn C, Hester A, Czogalla B, Mahner S, Rottmann M, Mayr D, Schmoeckel E, Trillsch F: The G-protein-coupled estrogen receptor (GPER) regulates trimethylation of Histone H3 at Lysine 4 and represses migration and proliferation of ovarian cancer cells in vitro. Cells 10(3): 619, 2021. DOI: 10.3390/cells10030619
26 Girgert R, Emons G, Gründker C: Inhibition of GPR30 by estriol prevents growth stimulation of triple-negative breast cancer cells by 17β-estradiol. BMC Cancer 14: 935, 2014. DOI: 10.1186/1471-2407-14-935
27 Mo Z, Liu M, Yang F, Luo H, Li Z, Tu G, Yang G: GPR30 as an initiator of tamoxifen resistance in hormone-dependent breast cancer. Breast Cancer Res 15(6): R114, 2013. DOI: 10.1186/bcr3581
28 Hopkins DR, Keles S, Greenspan DS: The bone morphogenetic protein 1/Tolloid-like metalloproteinases. Matrix Biol 26(7): 508-523, 2007. DOI: 10.1016/j.matbio.2007.05.004
29 Lepucki A, Orlińska K, Mielczarek-Palacz A, Kabut J, Olczyk P, Komosińska-Vassev K: The role of extracellular matrix proteins in breast cancer. J Clin Med 11(5): 1250, 2022. DOI: 10.3390/jcm11051250
30 Kobayashi H, Gieniec KA, Wright JA, Wang T, Asai N, Mizutani Y, Lida T, Ando R, Suzuki N, Lannagan TRM, Ng JQ, Hara A, Shiraki Y, Mii S, Ichinose M, Vrbanac L, Lawrence MJ, Sammour T, Uehara K, Davies G, Lisowski L, Alexander IE, Hayakawa Y, Butler LM, Zannettino ACW, Din MO, Hasty J, Burt AD, Leedham SJ, Rustgi AK, Mukherjee S, Wang TC, Enomoto A, Takahashi M, Worthley DL, Woods SL: The balance of stromal BMP signaling mediated by GREM1 and ISLR drives colorectal carcinogenesis. Gastroenterology 160(4): 1224-1239.e30, 2021. DOI: 10.1053/j.gastro.2020.11.011
31 Erin N, Tavşan E, Haksever S, Yerlikaya A, Riganti C: Targeting BMP-1 enhances anti-tumoral effects of doxorubicin in metastatic mammary cancer: common and distinct features of TGF-β inhibition. Breast Cancer Res Treat 210(3): 563-574, 2025. DOI: 10.1007/s10549-024-07592-4
32 Wang X, Eichhorn PJA, Thiery JP: TGF-β, EMT, and resistance to anti-cancer treatment. Semin Cancer Biol 97: 1-11, 2023. DOI: 10.1016/j.semcancer.2023.10.004
33 Maggiolini M, Picard D: The unfolding stories of GPR30, a new membrane-bound estrogen receptor. J Endocrinol 204(2): 105-114, 2010. DOI: 10.1677/JOE-09-0242
34 Marqués G, Musacchio M, Shimell MJ, Wünnenberg-Stapleton K, Cho KW, O'Connor MB: Production of a DPP activity gradient in the early Drosophila embryo through the opposing actions of the SOG and TLD proteins. Cell 91(3): 417-426, 1997. DOI: 10.1016/s0092-8674(00)80425-0
35 Liu M, Sui L, Fang Z, Jiang WG, Ye L: Aberrant expression of bone morphogenetic proteins in the disease progression and metastasis of breast cancer. Front Oncol 13: 1166955, 2023. DOI: 10.3389/fonc.2023.1166955
36 Prossnitz ER, Arterburn JB: International Union of Basic and Clinical Pharmacology. XCVII. G protein-coupled estrogen receptor and its pharmacologic modulators. Pharmacol Rev 67(3): 505-540, 2015. DOI: 10.1124/pr.114.009712

37 Pal U, Sahu A, Barah P, Limaye AM: Transcriptomic data of MCF-7 breast cancer cells treated with G1, a G-protein coupled estrogen receptor (GPER) agonist. Data Brief 41: 107948, 2022. DOI: 10.1016/j.dib.2022.107948
38 Günthert AR, Gründker C, Olota A, Läsche J, Eicke N, Emons G: Analogs of GnRH-I and GnRH-II inhibit epidermal growth factor-induced signal transduction and resensitize resistant human breast cancer cells to 4OH-tamoxifen. Eur J Endocrinol 153(4): 613-625, 2005. DOI: 10.1530/eje.1.01996
39 Von Alten J, Fister S, Schulz H, Viereck V, Frosch K, Emons G, Gründker C: GnRH analogs reduce invasiveness of human breast cancer cells. Breast Cancer Res Treat 100(1): 13-21, 2006. DOI: 10.1007/s10549-006-9222-z
40 Anastasi C, Rousselle P, Talantikite M, Tessier A, Cluzel C, Bachmann A, Mariano N, Dussoyer M, Alcaraz LB, Fortin L, Aubert A, Delolme F, El Kholti N, Armengaud J, Fournié P, Auxenfans C, Valcourt U, Goff SV, Moali C: BMP-1 disrupts cell adhesion and enhances TGF-β activation through cleavage of the matricellular protein thrombospondin-1. Sci Signal 13(639): eaba3880, 2020. DOI: 10.1126/scisignal.aba3880
41 Cosentino G, Plantamura I, Tagliabue E, Iorio MV, Cataldo A: Breast cancer drug resistance: overcoming the challenge by capitalizing on microRNA and tumor microenvironment interplay. Cancers (Basel) 13(15): 3691, 2021. DOI: 10.3390/cancers13153691
42 Liu M, Koo D, Jiang WG, Ye L: Oestrogen represses noggin expression by interfering with BMP/Smad signalling in ER positive breast cancer. Anticancer Res 44(8): 3355-3364, 2024. DOI: 10.21873/anticanres.17156
43 Huang G, Zhang Y, Kim B, Ge G, Annis DS, Mosher DF, Greenspan DS: Fibronectin binds and enhances the activity of bone morphogenetic protein 1. J Biol Chem 284(38): 25879-25888, 2009. DOI: 10.1074/jbc.M109.024125
44 Takeyama H, Manome Y, Koyama M, Yoshii Y, Taguchi E, Fuke A, Yamashita T, Fuse Y, Fushimi A, Nogi H: Sialic acid-fibronectin-negative and vimentin-positive breast cancers have poor prognoses due to epithelial-mesenchymal transition. Anticancer Res 45(4): 1617-1629, 2025. DOI: 10.21873/anticanres.17543