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Targeting G-coupled estrogen receptor signaling in melanoma

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Abstract: Melanoma is the most common malignancy diagnosed in pregnant woman. Some clinical data suggest the rapid progression of melanoma disease during the pregnancy.
It is well known that melanoma is the most immuno-dependent malignancy and during the pregnancy the immune system undergoes changes that can impact on tumor surveillance. However, clinical data on melanoma indicate the potential impact of sex hormones (especially estrogens) on progression of the melanoma disease and it is suggested that melanoma can be hormone dependent tumor.
In this paper, based on our experimental results, we focus on the role of G-protein coupled specific estrogen receptor (GPER) in melanoma. GPER receptor is the third type estrogen receptor with poorly understood function in melanoma. We show that GPER is expressed in MeWo melanoma cells. We also describe the effects of agonist G-1 and antagonist G15 of GPER as well as GPER siRNA silencing on proliferation of MeWo human melanoma cells. Proliferation of MeWo melanoma cells is reduced under activation of GPER dependent signaling. The presented data show a novel impact of GPER dependent signaling on the surveillance of melanoma.
Keywords: GPER, agonist G-1, antagonist G-15, melanoma, MeWo

1. Introduction

Melanoma malignant has been considered to be a hormone-related cancer since 1950s. Then, the potential influence of pregnancy on melanoma progression was mentioned for the first time [1]. Results of only few studies regarding cancer incidence during pregnancy are available and according to them melanoma is one of the most commonly diagnosed malignancies during pregnancy [2, 3]. Interestingly melanoma is also much more frequent in women than in men in age group from 20 to 50 years old, during the period when sex hormone level differ greatly between females and males [4]. The hypothesis that melanoma cells benefit from hormonal changes e.g. from increased serum plasma levels of estradiol was taken into account and although many studies on influence of estradiol on melanoma growth were performed, results of them are contradictory. Some of researchers showed that estradiol has no influence on melanoma proliferation [5], few of them report that estrogens inhibit melanoma growth [6] and the other suggest that estradiol induces proliferation of melanoma cells *in vitro* [7]. Thus, the role of estradiol in melanoma progression appears to be controversial. It is assumed that differences in melanoma response to estradiol depend on a presence or absence of estrogen receptors. Presence of intracellular receptors for estrogens ERα and ERβ in human primary melanomas were reported by few investigators [8÷9]. To our knowledge there are no available data from the studies on a presence of another estrogen receptor, a membrane G-protein coupled estrogen receptor (GPER formerly GPR30) in melanoma cells. GPER mediates rapid estradiol-induced nongenomic signaling and participates in the proliferative response in various cancer cell lines including endometrial, breast or ovarian cancer cell lines [10÷12]. In the present study we aimed to find out if the G-protein coupled receptor was present in the MeWo melanoma cells. We also studied influence of the GPER specific agonist G-1 and antagonist G-15 on melanoma cells viability.

2. Material and methods

2.1. Reagents

G-1 and G-15 were purchased from Tocris Bioscience and dissolved in DMSO as 10 mg/ml stock solution. 17-β estradiol was purchased from SIGMA Aldrich and dissolved as 5 mg/ml stock solution. Rabbit IgG polyclonal anti GPER antibody was purchased from Santa Cruz Biotechnology.

2.2. Cell lines and culture conditions

The human skin melanoma cell line MeWo and the human cervical cancer cell line HeLa was purchased from ATCC.

Both cell lines were maintained in vitro as monolayer cultures in RPMI 1640 medium (Biochrom, Germany) supplemented with 10% fetal calf serum (FCS, Biochrom, Germany) and antibiotic/antimycotic solution (Sigma-Aldrich) at 37°C in a humidified atmosphere of 5% CO_2. Cells were passaged every three days after washing with PBS and detached with trypsin/EDTA solution (all from Biochrom, Germany)

2.3. Transfection

For the siRNA transfection, cells were cultured in a six well culture plate until 80% of confluence was attained. Then the cells were incubated for 7 hours in previously prepared Transfection Reagent and Transfection Medium mixture (Santa Cruz) containing siRNA against GPER or control siRNA (Santa Cruz). Afterward cells were cultured for three days in normal growth medium containing antibiotics and 10% FCS and then used for further experiments including cells viability assays and immuno-fluorescence.

2.4. Western Blot

MeWo cell line was cultured in media enriched in 17-β estradiol at concentration 100 pg/ml for 72 hours or in media with vehicle (dimethylosulfoxide, DMSO). HeLa cell line which served as positive control was cultured in growth medium. Then lysates of cells were prepared using RIPA buffer (Sigma Aldrich) supplemented with protease and phosphatase inhibitors (Complete, Roche). Total protein concentration of each lysate sample was determined using BCA assay (Sigma-Aldrich). Equal amounts of protein was mixed with sample buffer and then heated at 95°C for 5 minutes. Electrophoresis was conducted on 0.1% SDS-10% polyacrylamide gel for one hour and next separated proteins were transferred to PFVD membrane (BioRad). Membrane was blocked with 3% skimmed milk for one hour in room temperature and incubated overnight at 4°C with diluted (1:200) anti GPER antibody or anti Rab11 rabbit polyclonal antibody (Santa Cruz). Membrane was washed tree times with TBS and incubated in 5% blocking buffer containing diluted goat anti rabbit HRP-conjugated antibodies for one hour. Signals were detected on X- ray films by means of chemi-luminescence. Relative bands density measurements was performed with ImageJ software.

2.5. Immunofluorescence

For the immunofluorescence experiments, cultured cells were seeded in microscope chamber slides and allowed to attach for 24 h. Then the cells were fixed with buffered 4% paraformaldehyde, permeabilized with PBS 0.2% triton X-100 and incubated in bovine serum albumin 5% to block nonspecific binding. Immunocytochemistry staining was performed using rabbit polyclonal anti-GPER antibody (Santa Cruz, dilution 1:500) Incubation with the primary antibody was performed at 4°C overnight. After washing 3-times in PBS the chamber-slides were incubated in the secondary anti-rabbit antibody Alexa Fluor 488 (Invitrogen, dilution 1:1000) for one hour, washed 3-times in PBS and mounted in Vectashield containing4′,6-diamidino-2-phenylindole (DAPI) (Vectorlabs, USA). Images were captured by Leica confocal microscope and analyzed by means of Las AF software (Leica, Germany).

2.6. Proliferation assays

For proliferation assays we used cells cultured in regular media or cells transfected with siRNA silencing GPER gene. 5 000 cells per well were seeded on 96 well plates and left to attach. After 24 hours media in wells were replaced with media enriched with G-1 or G-15 at concentrations from 1 nM to 10 μM. Cells treated with vehicle (DMSO) were used as a controls. After 48 hours cells viability assays using Presto Blue reagent according to manufacturer protocol were conducted to determine cell counts.

3. Results

3.1. GPER receptor is expressed in human melanoma cell line MeWo cells and is silenced by si-RNA

The expression of GPER protein in MeWo cell line was demonstrated by means of western blot using anti GPER antibody (Santa Cruz) (Figure 1 a). The densitometric analysis by ImageJ software showed that GPER is expressed in MeWo melanoma cells at lower level than in HeLa human cervical cancer cells, which served us as positive control (Figure 1 b).

We also analyzed the influence of 17-β-estradiol (E2) on GPER expression. As it is shown in Fig. 1b., the relative density of GPER band is similar in the presence and absence of 17-β estradiol both, suggesting no significant effect of 17-β estradiol on GPER expression.

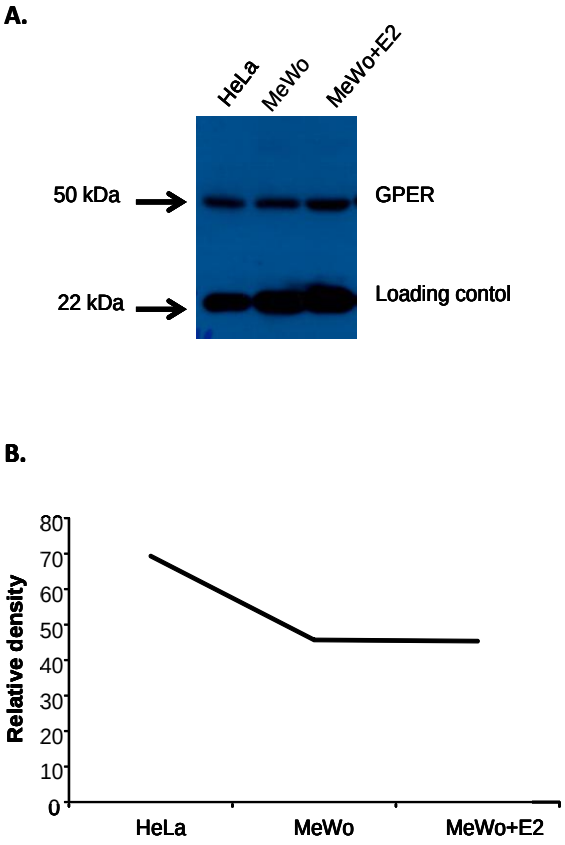

Figure 1. The protein expression of GPER was demonstrated by using Western blotting. A, Western blotting bands. MeWo cells were treated with vehicle or E2 (100pg/ml). HeLa cell line treated previously with vehicle served as positive control. B, Optical bands density analysis. Each sample was normalized to loading control content. GPER is expressed at lower level in MeWo cells than in HeLa cells. The expression is not changed by the E2 stimulation

Moreover, we also performed immuno-fluorescence staining to confirm GPER expression in MeWo melanoma cells.

The MeWo cells are positive for GPER and its immunoreactivity give strong signal in all of the cells. The signal was detected in the cytoplasm confirming its intercellular membrane localization (mainly in ER membranes) as well as at the cell surface (Figure 2). Moreover, the intensity of the observed GPER signal was reduced in cells under siRNA silencing of GPER (data not shown). This observation confirms the specificity of the used antibodies.

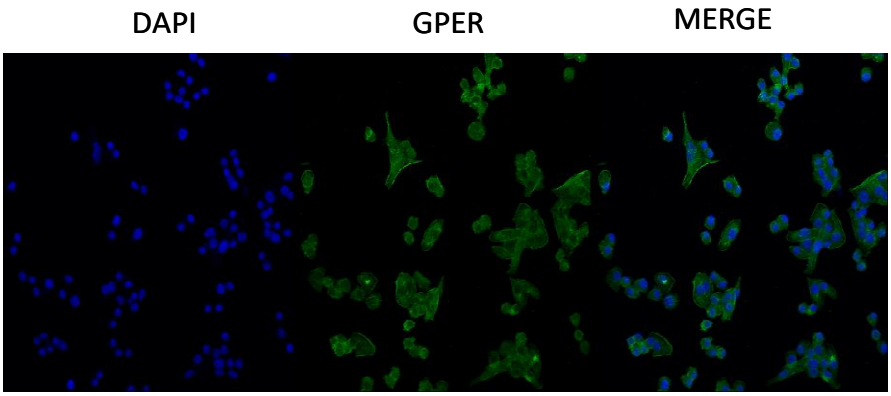

Figure 2. MeWo cells were positive for GPER (green).

Immunofluorescence staining of MeWo cells. Left panel: cell nuclei stained with DAPI. Middle panel: GPER expression at cell surface and within the cytoplasm. Right panel: overlay of DAPI and GPER signals

3.2. GPER agonist reduce the number of viable MeWo melanoma cells, while antagonist had no such effect

We studied the effect of GPER agonist and antagonist on MeWo melanoma cells viability.

The GPER activation or inhibition was performed using its agonist G-1 and antagonist G-15 and the influence of them on MeWo melanoma cells was investigated.

For GPER activation or inhibition its agonist and antagonist were used and the viability was assessed after 48 hours of incubation with agonist or antagonist.

After treatment with G1 agonist (in concentrations from 1- to 10 μM) the number of viable cells was significantly lower as it is shown in Figure 3.

In the group treated with G-1 antagonist at 1μM the number of viable cells was 63% ($p<0.05$) in comparison to the control. Treatment with G-1 antagonist at concentration of 10 μm caused maximum reduction of cells viability almost to 48% of control ($p<0.05$).

The GPER antagonist G15 alone did not reduce the number of melanoma cells significantly when compared to the control (data not shown).

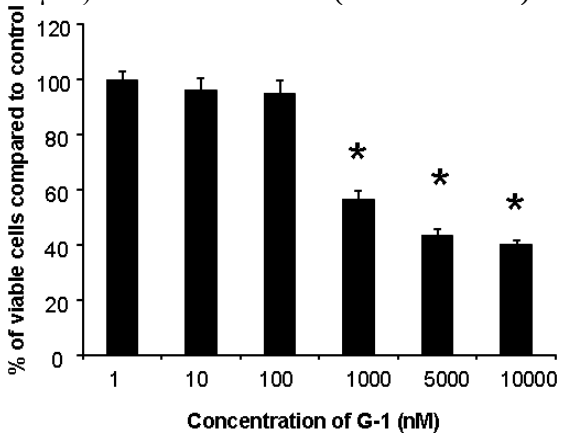

Figure 3. G-1 reduced the viability of MeWo cells. MeWo cells were treated with G-1 (1nM-10μM) or vehicle (DMSO) and after 48 hours of exposure the viability was determined by Presto Blue assay. Results are expressed as mean ± SEM and statistical significance ($p\leq0.05$) was assessed by Student T-test

4. Discussion and review

4.1. GPER – general data

Based on epidemiological data there exist a hypothesis that melanoma is a hormone, esp. estrogen, dependent cancer. However, there are only few reports in the literature regarding the presence of the estrogen receptors in melanoma cells and most of them refer to nuclear receptors ERα and ERβ alone [5, 6]. There is no specific information available on the presence of the estrogen membrane receptor- GPER in melanoma. However the upregulation of GPER was shown to stimulate melanin production and melanogenesis by protein kinase A (PKA) pathway [13].

Thus our paper focuses on surface receptor for estrogen (GPER) that mediates immediate estrogen effect. In the present study we demonstrated for the first time that GPER is expressed at protein level in human melanoma cell line – MeWo.

4.2. GPER – receptor

Membrane estrogen receptor named as GPER mediates rapid, nongenomic reaction of cells that are estrogen sensitive. The previous name for this receptor was GPR30. This receptor belongs to the seven domain transmembrane receptor family and is coupled with G-protein. Identification of GPER agonists and antagonists enabled studies on estrogen dependent signaling in physiology and pathology of sensitive cells and tissues [13÷15].

4.3. GPER – localization

Membrane bounded receptor can be localized in the cell membrane, or in any intracellular membrane-enclosed compartment. Also receptor recirculation from compartment to compartment is possible. Based on the literature, GPER receptor undergoes also its specific translocation.

First it was published that GPER is localized in the ER membranes [16], however another investigators reported its cell membrane localization [17].

The GPER receptor was shown to be localized in the cell membrane in HEK-293 cells, hippocampal CA-2 cells, ovaries of *Micropogonias undulates*, murine oocytes, myometrium [18÷22].

While cytoplasmatic/ER/Golgi localization has been shown for: COS7, CHO, HEK-293, HEC-50, MDA-MB231, H-38, hippocampal neurons, rat spermatocytes [23÷28].

Some authors suggest also the possibility of nuclear GPER localization in HUVEC and CAF cells [29÷32].

Our data show both cell membrane and cytoplasmatic GPER localization (Figure 2). The stronger signal was observed form the plasma membrane, the slighter came from cytoplasmatic structures. This observation can be easily explained by the well known fact that cell membranes circulate constantly between ER and plasma membrane in vesicles transporting phospholipids and proteins to the plasma membrane. The suggested ER localization give many unsolved questions about signaling cascade esp. localization of G-protein (inner or outer face), thus plasma membrane localization seems to be more physiological. Moreover, despite the fact of different cellular localizations of GPER it is still unknown in which of then the receptor is functionally active.

4.4. GPER receptor signaling

In the classical estrogen signaling, receptors for ligands are localized within the cell and act as transcription factors. However, this signal transduction mechanism does not explain rapid changes in cell metabolism after estrogen exposition.

The nongenomic action of estrogens is mediated by membrane bound receptor (GPER) discovered in year 2000 [33]. GPER is a G-coupled receptor and transduces signal using G-protein. In classical pathway it activates adenylyl cyclase and thereby protein kinase A [15, 17, 19, 34÷35]. PKA activation causes both fast metabolic effect as well as long term transcriptional gene activation by cAMP response element binding protein (CREB) activation [36÷38].

It was shown that GPER activates also MAP kinase pathway (MAPK), ERK (extracellular signal-regulated kinase) by Ras/Raf pathway as well and phosphoinositide 3-kinase (PI3K), enabling Akt (known as protein kinase B-PKB) kinase activation. In addition, activation of phospholipase C (PLC) leads to IP3/Calcium dependent signaling [16, 19, 39÷42].

The Ras/Raf/Erk, PI3K/Akt, and MAPK pathways are the main cellular regulators of cell growth and proliferation, and Raf is a common molecular target for melanoma treatment nowadays [43]. Thereby activation of GPER in melanoma triggers the critical pathways for cell surveillance and can be potentially good candidate for drug targeting.

In the contrast to direct G-protein dependent PKA and PLC signaling, the activation of these pathways recruits cross-activation of epidermal growth factor receptor (EGFR), that takes place by activation of Src kinase leading to MMP dependent releasing of heparin-binding EGF-like growth factor (HB-EGF) form its membrane bound form. HB-EGF binds to EGF receptor activating mainly Ras/Raf/Erk pathway, however PI3K/Akt and MAPK pathways can be also triggered [33, 41].

It was also shown that GPER stimulation by 17β-estradiol leads to activation of another pathway closely related to cell growth and differentiation – Notch [44].

Summarizing the GPER dependent signaling is very wide and involves many crucial pathways for melanoma cell surveillance.

4.5. GPER – and tumor

In our study we show for the first time the antiproliferative effect of GPER agonist G1 in melanoma MeWo cells. G1 agonist demonstrates antimelanoma activity at concentration starting from 1 μM. Recently, G1 agonist was also shown to reduce the viability of prostate cancer cells [45]. Moreover, the deficiency of GPER resulted in increased tumorigenesis in the liver [46].

The best characterized in the literature is the effect of GPER stimulation in ovarian, breast and prostate cancer that represent hormone-dependent malignances.

Activation of GPER in prostate cancer cells leads to growth inhibition and G2/M cell cycle arrest acting by Erk1/2 pathway and p21 upregulation. The inhibitory effect was observed in both androgen-dependent and androgen-independent prostate cancer cells *in vitro* and *in vivo* [45, 47].

In ovarian cancer cells GPER agonist G1 was shown to block tubulin poly-merization [48] and to inhibit cell proliferation by inducing G2/M cell cycle arrest and to initiate caspase-dependent apoptosis [49]. Moreover, in the same study Ignatov et al. show that GPER expression in ovarian cancers clinically correlates with higher 2-year disease-free survival of patients (28.6% for GPER-1 negative and 59.2% for GPER-1 positive cases by $p = 0.002$). This observation makes GPER positive ovarian cancer potential candidate for GPER targeted therapy.

Also in breast cancer cells G1 GPER agonist displayed anticancer activity inducing G2/M cell cycle arrest [50]. However, the data on the effect of GPER activation/ inhibition in breast cancer cells are contradictory, it was also shown that inhibition of this receptor by natural estriol (not synthetic G1 agonist) inhibits the growth of triple-negative breast cancer cells [51]. Moreover, Albanito et al. demonstrated that G-1 can potentially simulate the proliferation of ovarian and breast cancer cell lines [52]. These contradictory observations taken together with postulated non-receptor action of G1 [53] should turn us for a detailed research before potential clinical usage of GPER targeted therapy.

It is worth to mention that antagonists/modulators of canonical estrogen like tamoxifen, commonly used in the treatment of breast cancer was also shown to display GPER agonist activity [14].

G1 GPER agonist acts also on non-hormone dependent cancers and noncancerous cells e.g. it inhibits antiproliferative action of human vascular smooth muscle cells [54] or mediates progression of NSCLC (non small cell lung cancer).

Nowadays, according to GPER discovery in melanoma, the old question of possible estrogen targeting in melanoma is re-opend [55]. Recently, GPER agonist was shown to stimulate melanin production in melanoma cells [13], and here we describe its antimelamona activity. Thus G-1 GPER agonist should be considered as a new promising drug for cancer treatments including the therapy of melanoma.

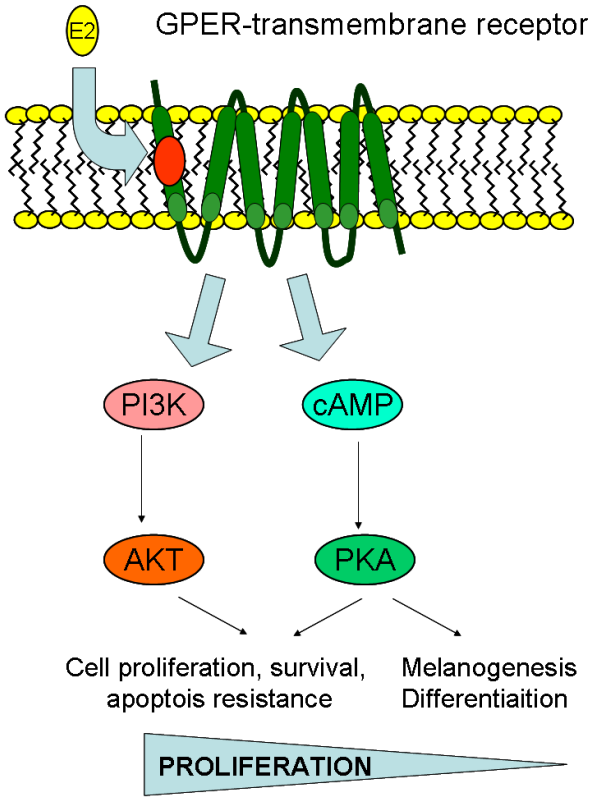

Figure 4. The effects of GPER activation on the proliferation of melanoma cells depends on balance in signaling pathways. E2 – estrogen as ligand, PI3K – Phosphoinositide 3-kinase, AKT – known also protein kinase B, cAMP – cyclic adenosine monophosphate, PKA – protein kinase A. Based on [14÷15].

5. Summary

In our study we showed for the first time that GPER receptor is expressed in human melanoma cells and GPER stimulation by its G-1 agonist reduces viability of melanoma cells. Thus, we propose GPER receptor to be a good candidate for research and targeting in antimelanoma strategies.

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Literature

1. Pack G. T.,Scharnagel I. M., *The Prognosis for Malignant Melanoma in the Pregnant Woman*, Cancer, 4 (1951), s. 324-334
2. Van Calsteren K., Heyns L., De Smet F., Van Eycken L., Gziri M. M., Van Gemert W., Halaska M., Vergote I., Ottevanger N.,Amant F., *Cancer During Pregnancy: An Analysis of 215 Patients Emphasizing the Obstetrical and the Neonatal Outcomes*, Journal of Clinical Oncology, 28 (2010), s. 683-689
3. Peccatori F. A., Azim H. A., Jr., Orecchia R., Hoekstra H. J., Pavlidis N., Kesic V., Pentheroudakis G.,Group E. G. W. *Cancer, Pregnancy and Fertility: Esmo Clinical Practice Guidelines for Diagnosis, Treatment and Follow-Up*, Annals of Oncology, 24 Suppl 6 (2013), s. vi160-170
4. *Http://Www.Cancerresearchuk.Org/Health-Professional/Cancer-Statistics/Statistics-by-Cancer-Type/Skin-Cancer/Mortality*, Cancer Research UK. Malignant Melanoma (C43), Average Number of New Cases per Year and Age-Specific Incidence Rates per 100,000 Population, UK, 2009-2011
5. Feucht K. A., Walker M. J., Das Gupta T. K.,Beattie C. W., *Effect of 17 Beta-Estradiol on the Growth of Estrogen Receptor-Positive Human Melanoma in Vitro and in Athymic Mice*, Cancer research, 48 (1988), s. 7093-7101
6. Sarti M. S., Visconti M. A.,Castrucci A. M., *Biological Activity and Binding of Estradiol to Sk-Mel 23 Human Melanoma Cells*, Brazilian Journal of Medical and Biological Research, 37 (2004), s. 901-905

7. Richardson B., Price A., Wagner M., Williams V., Lorigan P., Browne S., Miller J. G.,Mac Neil S., *Investigation of Female Survival Benefit in Metastatic Melanoma*, British Journal of Cancer, 80 (1999), s. 2025-2033
8. de Giorgi V., Mavilia C., Massi D., Gozzini A., Aragona P., Tanini A., Sestini S., Paglierani M., Boddi V., Brandi M. L.,Lotti T., *Estrogen Receptor Expression in Cutaneous Melanoma: A Real-Time Reverse Transcriptase-Polymerase Chain Reaction and Immunohistochemical Study*, Archives of Dermatology, 145 (2009), s. 30-36
9. Marzagalli M., Casati L., Moretti R. M., Montagnani Marelli M.,Limonta P., *Estrogen Receptor Beta Agonists Differentially Affect the Growth of Human Melanoma Cell Lines*, PLoS One, 10 (2015), s. e0134396
10. Skrzypczak M., Schuler S., Lattrich C., Ignatov A., Ortmann O.,Treeck O., *G Protein-Coupled Estrogen Receptor (Gper) Expression in Endometrial Adenocarcinoma and Effect of Agonist G-1 on Growth of Endometrial Adenocarcinoma Cell Lines*, Steroids, 78 (2013), s. 1087-1091
11. Liu H., Yan Y., Wen H., Jiang X., Cao X., Zhang G.,Liu G., A *Novel Estrogen Receptor Gper Mediates Proliferation Induced by 17beta-Estradiol and Selective Gper Agonist G-1 in Estrogen Receptor Alpha (Eralpha)-Negative Ovarian Cancer Cells*, Cell Biology International, 38 (2014), s. 631-638
12. Scaling A. L., Prossnitz E. R.,Hathaway H. J., *Gper Mediates Estrogen-Induced Signaling and Proliferation in Human Breast Epithelial Cells and Normal and Malignant Breast*, Hormones and Cancer, 5 (2014), s. 146-160
13. Sun M., Xie H. F., Tang Y., Lin S. Q., Li J. M., Sun S. N., Hu X. L., Huang Y. X., Shi W.,Jian D., *G Protein-Coupled Estrogen Receptor Enhances Melanogenesis Via Camp-Protein Kinase (Pka) by Upregulating Microphthalmia-Related Transcription Factor-Tyrosinase in Melanoma*, The Journal of steroid biochemistry and molecular biology, (2016)
14. Jacenik D., Cygankiewicz A. I.,Krajewska W. M., *The G Protein-Coupled Estrogen Receptor as a Modulator of Neoplastic Transformation*, Molecular and cellular endocrinology, 429 (2016), s. 10-18
15. Cygankiewicz A. I., Jacenik D.,Krajewska W. M., *[Gper Receptor – the New Player in Estrogen Signaling]*, Postepy Biochemii, 61 (2015), s. 52-60
16. Revankar C. M., Cimino D. F., Sklar L. A., Arterburn J. B.,Prossnitz E. R., A *Transmembrane Intracellular Estrogen Receptor Mediates Rapid Cell Signaling*, Science, 307 (2005), s. 1625-1630
17. Thomas P., Pang Y., Filardo E. J.,Dong J., *Identity of an Estrogen Membrane Receptor Coupled to a G Protein in Human Breast Cancer Cells*, Endocrinology, 146 (2005), s. 624-632
18. Rinne T., Spadoni E., Kjaer K. W., Danesino C., Larizza D., Kock M., Huoponen K., Savontaus M. L., Aaltonen M., Duijf P., Brunner H. G., Penttinen M.,van Bokhoven H., *Delineation of the Adult Syndrome Phenotype Due to Arginine 298 Mutations of the P63 Gene*, European Journal of Human Genetics, 14 (2006), s. 904-910
19. Filardo E., Quinn J., Pang Y., Graeber C., Shaw S., Dong J.,Thomas P., *Activation of the Novel Estrogen Receptor G Protein-Coupled Receptor 30 (Gpr30) at the Plasma Membrane*, Endocrinology, 148 (2007), s. 3236-3245
20. Pang Y., Dong J.,Thomas P., *Estrogen Signaling Characteristics of Atlantic Croaker G Protein-Coupled Receptor 30 (Gpr30) and Evidence It Is Involved in Maintenance of Oocyte Meiotic Arrest*, Endocrinology, 149 (2008), s. 3410-3426
21. Lenhart P. M., Broselid S., Barrick C. J., Leeb-Lundberg L. M.,Caron K. M., *G-Protein-Coupled Receptor 30 Interacts with Receptor Activity-Modifying Protein 3 and Confers Sex-Dependent Cardioprotection*, Journal of Molecular Endocrinology, 51 (2013), s. 191-202
22. Li Y. R., Ren C. E., Zhang Q., Li J. C.,Chian R. C., *Expression of G Protein Estrogen Receptor (Gper) on Membrane of Mouse Oocytes During Maturation*, Journal of Assisted Reproduction and Genetics, 30 (2013), s. 227-232
23. Maiti K., Paul J. W., Read M., Chan E. C., Riley S. C., Nahar P.,Smith R., *G-1-Activated Membrane Estrogen Receptors Mediate Increased Contractility of the Human Myometrium*, Endocrinology, 152 (2011), s. 2448-2455
24. Wang C., Prossnitz E. R.,Roy S. K., *G Protein-Coupled Receptor 30 Expression Is Required for Estrogen Stimulation of Primordial Follicle Formation in the Hamster Ovary*, Endocrinology, 149 (2008), s. 4452-4461
25. Otto C., Rohde-Schulz B., Schwarz G., Fuchs I., Klewer M., Brittain D., Langer G., Bader B., Prelle K., Nubbemeyer R.,Fritzemeier K. H., *G Protein-Coupled Receptor 30 Localizes to the Endoplasmic Reticulum and Is Not Activated by Estradiol*, Endocrinology, 149 (2008), s. 4846-4856
26. Lin B. C., Suzawa M., Blind R. D., Tobias S. C., Bulun S. E., Scanlan T. S.,Ingraham H. A., *Stimulating the Gpr30 Estrogen Receptor with a Novel Tamoxifen Analogue Activates Sf-1 and Promotes Endometrial Cell Proliferation*, Cancer research, 69 (2009), s. 5415-5423

27. Sakamoto H., Matsuda K., Hosokawa K., Nishi M., Morris J. F., Prossnitz E. R.,Kawata M., *Expression of G Protein-Coupled Receptor-30, a G Protein-Coupled Membrane Estrogen Receptor, in Oxytocin Neurons of the Rat Paraventricular and Supraoptic Nuclei*, Endocrinology, 148 (2007), s. 5842-5850
28. Matsuda K., Sakamoto H., Mori H., Hosokawa K., Kawamura A., Itose M., Nishi M., Prossnitz E. R.,Kawata M., *Expression and Intracellular Distribution of the G Protein-Coupled Receptor 30 in Rat Hippocampal Formation*, Neuroscience Letters, 441 (2008), s. 94-99
29. Chimento A., Sirianni R., Delalande C., Silandre D., Bois C., Ando S., Maggiolini M., Carreau S.,Pezzi V., *17 Beta-Estradiol Activates Rapid Signaling Pathways Involved in Rat Pachytene Spermatocytes Apoptosis through Gpr30 and Er Alpha*, Molecular and cellular endocrinology, 320 (2010), s. 136-144
30. Chakrabarti S.,Davidge S. T., *G-Protein Coupled Receptor 30 (Gpr30): A Novel Regulator of Endothelial Inflammation*, PLoS One, 7 (2012), s. e52357
31. Madeo A.,Maggiolini M., *Nuclear Alternate Estrogen Receptor Gpr30 Mediates 17beta-Estradiol-Induced Gene Expression and Migration in Breast Cancer-Associated Fibroblasts*, Cancer research, 70 (2010), s. 6036-6046
32. Pupo M., Vivacqua A., Perrotta I., Pisano A., Aquila S., Abonante S., Gasperi-Campani A., Pezzi V.,Maggiolini M., *The Nuclear Localization Signal Is Required for Nuclear Gper Translocation and Function in Breast Cancer-Associated Fibroblasts (Cafs)*, Molecular and cellular endocrinology, 376 (2013), s. 23-32
33. Filardo E. J., Quinn J. A., Bland K. I.,Frackelton A. R., Jr. *Estrogen-Induced Activation of Erk-1 and Erk-2 Requires the G Protein-Coupled Receptor Homolog, Gpr30, and Occurs Via Trans-Activation of the Epidermal Growth Factor Receptor through Release of Hb-Egf*, Mol Endocrinol, 14 (2000), s. 1649-1660
34. Filardo E. J., Quinn J. A., Frackelton A. R., Jr.,Bland K. I., *Estrogen Action Via the G Protein-Coupled Receptor, Gpr30: Stimulation of Adenylyl Cyclase and Camp-Mediated Attenuation of the Epidermal Growth Factor Receptor-to-Mapk Signaling Axis*, Mol Endocrinol, 16 (2002), s. 70-84
35. Thomas P.,Dong J., *Binding and Activation of the Seven-Transmembrane Estrogen Receptor Gpr30 by Environmental Estrogens: A Potential Novel Mechanism of Endocrine Disruption*, The Journal of steroid biochemistry and molecular biology, 102 (2006), s. 175-179
36. Kanda N.,Watanabe S., *17beta-Estradiol Stimulates the Growth of Human Keratinocytes by Inducing Cyclin D2 Expression*, J Invest Dermatol, 123 (2004), s. 319-328
37. Lindsey S. H., Liu L.,Chappell M. C., *Vasodilation by Gper in Mesenteric Arteries Involves Both Endothelial Nitric Oxide and Smooth Muscle Camp Signaling*, Steroids, 81 (2014), s. 99-102
38. Zucchetti A. E., Barosso I. R., Boaglio A. C., Basiglio C. L., Miszczuk G., Larocca M. C., Ruiz M. L., Davio C. A., Roma M. G., Crocenzi F. A.,Pozzi E. J., *G-Protein-Coupled Receptor 30/Adenylyl Cyclase/Protein Kinase a Pathway Is Involved in Estradiol 17ss-D-Glucuronide-Induced Cholestasis*, Hepatology, 59 (2014), s. 1016-1029
39. Ariazi E. A., Brailoiu E., Yerrum S., Shupp H. A., Slifker M. J., Cunliffe H. E., Black M. A., Donato A. L., Arterburn J. B., Oprea T. I., Prossnitz E. R., Dun N. J.,Jordan V. C., *The G Protein-Coupled Receptor Gpr30 Inhibits Proliferation of Estrogen Receptor-Positive Breast Cancer Cells*, Cancer research, 70 (2010), s. 1184-1194
40. Brailoiu E., Dun S. L., Brailoiu G. C., Mizuo K., Sklar L. A., Oprea T. I., Prossnitz E. R.,Dun N. J., *Distribution and Characterization of Estrogen Receptor G Protein-Coupled Receptor 30 in the Rat Central Nervous System*, J Endocrinol, 193 (2007), s. 311-321
41. Prossnitz E. R.,Maggiolini M., *Mechanisms of Estrogen Signaling and Gene Expression Via Gpr30*, Molecular and cellular endocrinology, 308 (2009), s. 32-38
42. Tica A. A., Dun E. C., Tica O. S., Gao X., Arterburn J. B., Brailoiu G. C., Oprea T. I.,Brailoiu E., *G Protein-Coupled Estrogen Receptor 1-Mediated Effects in the Rat Myometrium*, Am J Physiol Cell Physiol, 301 (2011), s. C1262-1269
43. Mandal R., Becker S.,Strebhardt K., *Stamping out Raf and Mek1/2 to Inhibit the Erk1/2 Pathway: An Emerging Threat to Anticancer Therapy*, Oncogene, 35 (2016), s. 2547-2561
44. Pupo M., Pisano A., Abonante S., Maggiolini M.,Musti A. M., *Gper Activates Notch Signaling in Breast Cancer Cells and Cancer-Associated Fibroblasts (Cafs)*, Int J Biochem Cell Biol, 46 (2014), s. 56-67
45. Laakmann E., Witzel I., Scriba V., Grzyska U., Zu Eulenburg C., Burchardi N., Hesse T., Wurschmidt F., Fehm T., Mobus V., von Minckwitz G., Loibl S., Park-Simon T. W.,Mueller V., *Radiological Patterns of Brain Metastases in Breast Cancer Patients: A Subproject of the German Brain Metastases in Breast Cancer (Bmbc) Registry*, Int J Mol Sci, 17 (2016)
46. Wei T., Chen W., Wen L., Zhang J., Zhang Q., Yang J., Liu H., Chen B. W., Zhou Y., Feng X., Yang Q., Bai X.,Liang T., *G Protein-Coupled Estrogen Receptor Deficiency Accelerates Liver Tumorigenesis by Enhancing Inflammation and Fibrosis*, Cancer letters, 382 (2016), s. 195-202
47. Chan Q. K., Lam H. M., Ng C. F., Lee A. Y., Chan E. S., Ng H. K., Ho S. M.,Lau K. M. *Activation of Gpr30 Inhibits the Growth of Prostate Cancer Cells through Sustained Activation of Erk1/2, C-Jun/C-*

Fos-Dependent Upregulation of P21, and Induction of G(2) Cell-Cycle Arrest, Cell Death Differ, 17 (2010), s. 1511-1523
48. Wang C., Lv X., He C., Hua G., Tsai M. Y.,Davis J. S., *The G-Protein-Coupled Estrogen Receptor Agonist G-1 Suppresses Proliferation of Ovarian Cancer Cells by Blocking Tubulin Polymerization*, Cell Death Dis, 4 (2013), s. e869
49. Ignatov T., Modl S., Thulig M., Weissenborn C., Treeck O., Ortmann O., Zenclussen A., Costa S. D., Kalinski T.,Ignatov A., *Gper-1 Acts as a Tumor Suppressor in Ovarian Cancer*, J Ovarian Res, 6 (2013), s. 51
50. Weissenborn C., Ignatov T., Poehlmann A., Wege A. K., Costa S. D., Zenclussen A. C.,Ignatov A., *Gper Functions as a Tumor Suppressor in Mcf-7 and Sk-Br-3 Breast Cancer Cells*, J Cancer Res Clin Oncol, 140 (2014), s. 663-671
51. Girgert R., Emons G.,Grundker C., *Inhibition of Gpr30 by Estriol Prevents Growth Stimulation of Triple-Negative Breast Cancer Cells by 17beta-Estradiol*, BMC Cancer, 14 (2014), s. 935
52. Albanito L., Madeo A., Lappano R., Vivacqua A., Rago V., Carpino A., Oprea T. I., Prossnitz E. R., Musti A. M., Ando S.,Maggiolini M., *G Protein-Coupled Receptor 30 (Gpr30) Mediates Gene Expression Changes and Growth Response to 17beta-Estradiol and Selective Gpr30 Ligand G-1 in Ovarian Cancer Cells*, Cancer research, 67 (2007), s. 1859-1866
53. Wang C., Lv X., Jiang C.,Davis J. S., *The Putative G-Protein Coupled Estrogen Receptor Agonist G-1 Suppresses Proliferation of Ovarian and Breast Cancer Cells in a Gper-Independent Manner*, American Journal of Translational Research, 4 (2012), s. 390-402
54. Gui Y., Shi Z., Wang Z., Li J. J., Xu C., Tian R., Song X., Walsh M. P., Li D., Gao J.,Zheng X. L., *The Gper Agonist G-1 Induces Mitotic Arrest and Apoptosis in Human Vascular Smooth Muscle Cells Independent of Gper*, Journal of Cellular Physiology, 230 (2015), s. 885-895
55. Ribeiro M. P., Santos A. E.,Custodio J. B., *Rethinking Tamoxifen in the Management of Melanoma: New Answers for an Old Question*, Eur J Pharmacol, 764 (2015), s. 372-378