The G Protein-Coupled Estrogen Receptor (GPER) Expression Correlates with Pro-Metastatic Pathways in ER-Negative Breast Cancer: A Bioinformatics Analysis

Marianna Talia 1,†, Ernestina Marianna De Francesco 2,†, Damiano Cosimo Rigiracciolo 1, Maria Grazia Muoio 1,2, Lucia Muglia 1, Antonino Belfiore 2, Marcello Maggiolini 1,* and Rosamaria Lappano 1

1 Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, 87036 Rende, Italy; marianna.talia@unical.it (M.T.); damianocosimo.rigiracciolo@unical.it (D.C.R.); mariagraziamuoio@libero.it (M.G.M.); lucia.muglia@unical.it (L.M.); rosamaria.lappano@unical.it (R.L.)

2 Endocrinology, Department of Clinical and Experimental Medicine, University of Catania, Garibaldi-Nesima Hospital, 95122 Catania, Italy; ernestinamarianna@yahoo.it (E.M.D.F.); antonino.belfiore@unict.it (A.B.)

3 MRC Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh EH4 2XR, UK

* Correspondence: marcellomaggiolini@yahoo.it (M.M.); andrew.sims@ed.ac.uk (A.H.S.)
† These authors contributed equally to this work.

Received: 5 February 2020; Accepted: 3 March 2020; Published: 4 March 2020

Abstract: The G protein-coupled estrogen receptor (GPER, formerly known as GPR30) is a seven-transmembrane receptor that mediates estrogen signals in both normal and malignant cells. In particular, GPER has been involved in the activation of diverse signaling pathways toward transcriptional and biological responses that characterize the progression of breast cancer (BC). In this context, a correlation between GPER expression and worse clinical-pathological features of BC has been suggested, although controversial data have also been reported. In order to better assess the biological significance of GPER in the aggressive estrogen receptor (ER)-negative BC, we performed a bioinformatics analysis using the information provided by The Invasive Breast Cancer Cohort of The Cancer Genome Atlas (TCGA) project and Molecular Taxonomy of Breast Cancer International Consortium (METABRIC) datasets. Gene expression correlation and the statistical analysis were carried out with R studio base functions and the tidyverse package. Pathway enrichment analysis was evaluated with Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway on the Database for Annotation, Visualization and Integrated Discovery (DAVID) website, whereas gene set enrichment analysis (GSEA) was performed with the R package phenoTest. The survival analysis was determined with the R package surviALL. Analyzing the expression data of more than 2500 primary BC, we ascertained that GPER levels are associated with pro-migratory and metastatic genes belonging to cell adhesion molecules (CAMs), extracellular matrix (ECM)-receptor interaction, and focal adhesion (FA) signaling pathways. Thereafter, evaluating the disease-free interval (DFI) in ER-negative BC patients, we found that the subjects expressing high GPER levels exhibited a shorter DFI in respect to those exhibiting low GPER levels. Overall, our results may pave the way to further dissect the network triggered by GPER in the breast malignancies lacking ER toward a better assessment of its prognostic significance and the action elicited in mediating the aggressive features of the aforementioned BC subtype.

Keywords: bioinformatics; GPER; breast cancer; TCGA; METABRIC; cell adhesion molecules; extracellular matrix; focal adhesion
1. Introduction

Breast cancer (BC) is the most frequently diagnosed tumor worldwide (24.2%) and the leading cause of cancer death among females (14.5%) [1]. The breast malignancies encompass diverse subtypes (Luminal A, Luminal B, Her2-enriched, Triple-negative/basal-like, and Normal-like) that are characterized by peculiar gene expression profiles, biological features, and clinical outcomes [2]. Estrogens play a pivotal role in numerous physiological conditions; however, the action of these steroids is also extensively associated with an increased risk of BC development [3]. The estrogen signaling is mainly mediated by the estrogen receptor (ER)$\alpha$ and ER$\beta$ that, upon ligand activation, regulate the expression of target genes involved in cell growth, invasion, and survival [4]. A growing body of data has also evidenced that the seven-transmembrane G protein-coupled estrogen receptor (GPER, previously known as GPR30) can mediate the estrogen action in diverse normal and malignant cell contexts, including BC [5]. The activation of GPER triggers diverse transduction pathways including the epidermal growth factor receptor (EGFR), phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt), and mitogen-activated protein kinases (MAPKs) toward transcriptional and biological responses driving the progression of BC [5–8]. In this regard, we recently found that GPER mediates the activation of the focal adhesion kinase (FAK) and the formation of focal adhesions (FAs) in triple negative BC cells (TNBC), thus contributing to the acquisition of aggressive features by breast malignancies [9]. In accordance with these data, immunohistochemical studies on breast tumors have shown that the expression of GPER is correlated with increased tumor size, distant metastasis, and recurrence [10,11]. Conversely, Martin and co-workers demonstrated that low expression levels of GPER are associated with aggressive features in a large cohort of primary invasive BC patients [12]. These controversial observations may deserve further investigations in order to better assess prognostic and therapeutic values of GPER, particularly in BC.

In the last years, high throughput techniques have been employed in handling and extracting meaningful information from large multiomic datasets [13]. In particular, big data analysis methods and classification techniques have allowed accurate and comprehensive examination of global gene expression profiles [14]. As cancer genomic datasets incessantly grow in terms of size and complexity, the availability of accessible computational resources may facilitate rapid and cost-effective analysis toward new discoveries in cancer biology and profiling [15]. The Cancer Genome Atlas (TCGA) project, initiated in 2005 by the National Cancer Institute, aims to collect genomic alterations implicated in cancer using genome analysis technologies [16]. Furthermore, the Molecular Taxonomy of Breast Cancer International Consortium (METABRIC) is a Canada-United Kingdom project integrating genomic and transcriptomic profiles of a large cohort of primary breast tumors (over two thousand) along with long-term clinical follow-up [17]. In particular, systematic advances in cancer genomics provided by both the TCGA and METABRIC databases have contributed to highlight similarities and differences in the genomic architecture of the breast malignancy as well as to identify new candidate biomarkers and drug tumor targets toward tailored therapeutic strategies [17–19]. Here, we focused on data extracted from both TCGA and METABRIC datasets in order to better understand the role exerted by GPER as well as its prognostic value in the aggressive BC subtype lacking ER. Indeed, our analysis provides new insights regarding the association of pro-metastatic pathways with GPER in the ER-negative BC, therefore opening a new scenario for subsequent studies aimed to better evaluate its role in breast tumors characterized by a worse prognosis.

2. Materials and Methods

2.1. Data Source

Data from the publically available TCGA and METABRIC datasets were used in the current study. mRNA expression data (RNA Seq V2 RSEM) and associated clinical information reported in the Invasive Breast Cancer Cohort of the TCGA project were retrieved on 4 November 2019 from cBioPortal for cancer genomics (http://www.cbioportal.org/) as well as microarray gene expression data (Log2 transformed intensity values) and clinical information of the METABRIC cohort. Patients of
both TCGA (N = 817) and METABRIC (N = 1980) were classified on the basis of the presence or the absence of ER (detected by immunohistochemistry). Gene expression and clinical information were filtered for missing values. The filtering resulted in 775 patients of TCGA and 1904 of METABRIC, which were used for the subsequent analysis.

2.2. Correlation Analysis

The Pearson correlation coefficients (r-values) between the expression levels of GPER and the other genes of the TCGA (N = 36877) and METABRIC (N = 24367) datasets were assessed in ER-negative BC patients using the cor.test() function and setting the method as “Pearson” in R Studio (version 3.6.1). The first 1000 most correlated genes of each dataset were intersected with the intersect() function in order to obtain the most correlated genes shared by the two datasets. The statistical analysis was performed by using the t-tests, and p < 0.001 was considered statistically significant.

2.3. Pathway Enrichment Analysis

In order to obtain the enrichment results of the selected genes into significant pathways, we uploaded our lists on the Database for Annotation, Visualization and Integrated Discovery (DAVID) functional annotation analysis website [20]. We analyzed a list of the 277 top GPER-correlated genes of the ER-negative patients shared by TCGA and METABRIC, choosing the official gene symbol as “select identifier” and gene list as “list type” in the options of the upload and selecting a limit species of “Homo sapiens” in the background. Selecting the functional annotation tool and the option of pathways, crucial pathways were obtained by KEGG pathway of DAVID and their numbers in the KEGG database.

2.4. Gene Set Enrichment Analysis (GSEA)

GSEA was performed using the gsea() function of the phenoTest package (https://bioconductor.org/packages/release/bioc/html/phenoTest.html) in R Studio in order to test the association between the predefined groups of genes and a specific phenotype. The gene lists used for this analysis, derived from DAVID functional annotation tool, are the CAMs pathway (KEGG entry = hsa04514), the ECM-receptor interaction pathway (KEGG entry = hsa04512), and the FA signaling pathway (KEGG entry = hsa04510). We ranked the genes in accordance with the differential expression within GPER high and low (median expression value as threshold assessment) samples in the ER-negative subgroup of BC patients, verifying if the selected set of genes were enriched at the bottom or the top of the ranked list. We calculated the enrichment score (ES) that reflects the degree to which a set of genes is overrepresented at the extremes (top or bottom) of the entire ranked list. The magnitude of the increment depends on the correlation of one gene with the phenotype. In this analysis, 20,000 simulations were used (B = 20,000). p < 0.05 was considered significant.

2.5. Survival Analysis

Comprehensive survival analysis was conducted using TCGA gene expression data of GPER along with the DFI information; patients were filtered for missing values, and the ER and the HER2 statuses were used to divide the population. The survALL package was employed to examine Cox proportional hazards for all possible points-of-separation (low-high cut-points), selecting the cut-point with the lowest p-value [21] and separating the patients into high (N = 27) and low (N = 93) GPER expression levels. The Kaplan–Meier survival curves were generated using the survival and the survminer packages.

2.6. Statistical Analysis

In this study, the analyses, including the t-test, and the scatter plots were performed with the R tydiverse package. p-values < 0.05 were considered significant. Heatmaps were performed with the R
The heatmap package. Gene expression values of both TCGA and METABRIC datasets were normalized by calculating their respective normalized z-scores.

3. Results

Considering that GPER-mediated signaling has been involved in BC development and aggressiveness [6,7,19], we began our study correlating the expression of GPER with the genes present in both TCGA and METABRIC datasets. In particular, we focused our investigations on ER-negative BC, as this malignancy is characterized by a worse prognosis [1,2,19]. To this end, we ranked the genes by Pearson correlation coefficient, assessing for the next evaluations the first 1000 genes positively correlated with GPER either in TCGA or METABRIC cohorts. Hence, we found 277 shared genes between the two datasets, as shown in Figure 1A and detailed in the Supplementary Table S1.

In order to investigate the biological significance of the aforementioned 277 genes, we then performed KEGG (The Kyoto Encyclopedia of Genes and Genomes) pathway analysis using the online Database for Annotation, Visualization and Integrated Discovery (DAVID, http://david.abcc.ncifcrf.gov). The 277 genes were enriched in a number of pathways, as schematically shown in Figure 1B. Of note, transduction pathways that characterize aggressive cancer features as cell adhesion molecules (CAMs), extracellular matrix (ECM)-receptor interaction, and focal adhesion (FA) appeared to be the most significant, as indicated by their respective -log10 adj p-value.

Figure 1. (A) Intersection of the top 1000 G protein-coupled estrogen receptor (GPER) correlated genes in estrogen receptor (ER)-negative breast cancer (BC) patients querying The Cancer Genome Atlas (TCGA) and Molecular Taxonomy of Breast Cancer International Consortium (METABRIC) datasets. (B) GPER expression is correlated with pro-metastatic pathways in ER-negative BC samples, as evaluated by The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of the 277 genes shared by the TCGA and METABRIC datasets and their positive correlation with GPER in ER-negative BC patients. The -log10 adjusted values are displayed along the x-axis, while the different KEGG pathways are shown along the y-axis. The number of the genes included in the identified pathways is plotted on the right of each bar.
In order to investigate the biological significance of the aforementioned 277 genes, we then performed KEGG (The Kyoto Encyclopedia of Genes and Genomes) pathway analysis using the online Database for Annotation, Visualization and Integrated Discovery (DAVID, http://david.abcc.ncifcrf.gov). The 277 genes were enriched in a number of pathways, as schematically shown in Figure 1B. Of note, transduction pathways that characterize aggressive cancer features as cell adhesion molecules (CAMs), extracellular matrix (ECM)-receptor interaction, and focal adhesion (FA) appeared to be the most significant, as indicated by their respective -log10 adj p-value.

Thereafter, we performed gene set enrichment analysis (GSEA) to explore the expression profile of the genes belonging to the CAMs, ECM-receptor interaction, and the FA pathways in the high and low GPER phenotypes of TCGA and METABRIC cohorts of ER-negative BC patients. It is worth noting that the genes included in these signaling pathways were found enriched in the group of patients showing high GPER levels (Supplementary Figure S1). In addition, we assessed the profile of the most GPER-correlated genes shared by the TCGA and METABRIC datasets. In this regard, we identified pro-tumorigenic genes belonging to the CAMs pathway as for instance the cell adhesion molecule 3 (CADM3), the CD34 molecule (CD34), the cadherin 5 (CDH5), the claudin 5 (CLDN5), the endothelial cell adhesion molecule (ESAM) and the junctional adhesion molecules namely JAM2 and JAM3 (Figure 2A). In addition, we evidenced further pro-tumorigenic genes belonging to the ECM-receptor interaction and FA pathways including for instance the caveolin 1 (CAV1), the alpha(α)1(VI) chain of type VI collagen (COL6A1 and COL6A2, respectively), the insulin like growth factor 1 (IGF1), the integrin subunit alpha 5 and the integrin subunit alpha 7 (ITGA5 and ITGA7, respectively), the laminin subunit beta 2 (LAMB2), the platelet derived growth factor receptor beta (PDGFRB), the placental growth factor (PGF) and the von Willebrand factor (VWF) (Figure 2B).

Figure 2. GPER correlates with the expression of cell adhesion molecules (CAMs), extracellular matrix (ECM)-receptor interaction, and focal adhesion (FA) pathway genes as determined querying the TCGA and the METABRIC datasets. The heatmaps, ranked from left to right, show the most GPER correlated genes belonging to the CAMs pathway (A) and to the ECM-receptor interaction and FA molecular pathways (B) in ER-negative breast tumor samples. Colors are z-score normalized values, red indicates high and blue indicates low.

Next, we evaluated whether the expression of GPER would be predictive for the outcome of the aggressive BC subtype characterized by the lack of both ER the human epidermal growth factor receptor 2 (HER2). Using the disease free interval (DFI) data, a significant cut-point was predictable only from the TCGA cohort. Ranking the gene expression data according to the low and high GPER levels, all possible points-of-separation and their significance were reported in the surviALL plot by
which the most significant cut-point was assessed (Figure 3A). Thereafter, the Kaplan-Meier survival curve revealed that a worse DFI characterizes the group of BC patients exhibiting a high expression of GPER (Figure 3B).

![Figure 3. Clinical outcome on the basis of GPER expression in ER-negative BC patients. (A) ER-negative BC patients of the TCGA cohort divided into high and low expression levels of GPER on the basis of the established cut-point. The color bar gradient stands for range of the most significant points-of-separation of the population (low-high significance = blue-yellow gradient) based on GPER expression and survival of each patient. The x-axis represents the patients ordered by the increasing expression of GPER. (B) Correlation between GPER expression and disease free interval (DFI) of ER-negative BC patients in the TCGA cohort.](image)

4. Discussion

The great amount of data on cancer-related molecular interactions and gene expression patterns has challenged the use of comprehensive information highlighting the multifaceted functions driving tumor progression. In this vein, large-scale informatics studies have provided the chance to handle and analyze the open-source biological datasets. As a better understanding of key regulatory networks involved in cancer biology may strongly boost the identification of new targets and innovative therapeutic approaches, the use of big data regarding the gene expression landscape in cohorts of cancer patients could represent a promising perspective. Considering the aforementioned remarks, the TCGA and the METABRIC datasets were queried to deepen the current knowledge on the action of GPER in BC development. Our data analysis demonstrated that a significant association of GPER with genes belonging to pro-migratory and metastatic signaling pathways occurs in the subset of BC patients lacking ER. Of note, these findings suggest that GPER may be involved in the metastatic dissemination of BC cells in the aforementioned patients. Finally, we ascertained that a worse DFI characterizes the subgroup of both ER and HER2-negative BC patients exhibiting a high expression of GPER, hence highlighting its potential role in the aggressive subtype of breast malignancies.

The action of GPER in mediating the stimulatory effects of estrogens in BC has been extensively reported [6,19,22,23]. In particular, it was established that GPER is involved in a complex transduction...
network that includes, for instance, the EGFR/MAPK signaling cascade, the adenyl cyclase, and PI3K, which in turn leads to gene expression changes and biological responses as the proliferation, the survival, and the migration of BC cells [5,6,24]. GPER and ER are considered to be unique estrogen receptors on the basis of their different chromosomal localization and the biochemical, the biological, and the pharmacological properties [19]. Worthy, the observations that BC cells lacking ER may express GPER and that the transcription of the two receptors is differentially regulated in BC phenotypes may indicate an independent beyond a cooperative action of GPER and ER in mediating the estrogen signaling [10,25,26]. In this respect, a physical and functional interaction occurring between these main transduction mediators was shown to regulate transcriptional and biological responses in cancer cells, hence indicating that GPER and ER may synergistically contribute to the malignant progression of estrogen-sensitive tumors [27–30]. Yet, numerous clinical studies have demonstrated that, in diverse cohorts of BC patients, high GPER levels are likely concomitant with an ER positivity [10,11,31,32]. Nevertheless, immunohistochemical analysis of 361 BC also revealed that the expression of GPER and ER may be not interdependent, as approximately 50% of ER-negative breast tumors retained GPER; therefore, GPER could drive estrogen responses in these peculiar cell contexts [10]. On the basis of these findings, we therefore focused on the gene expression profile and the signaling pathways associated with GPER in ER-negative BC patients. Of note, the expression of pro-metastatic CAMs, ECM-receptor interaction, and FA genes was found as the most correlated with GPER in this cell context, suggesting the potential of GPER to contribute to spreading and metastatic outgrowth of BC cells, as previously reported [7,19,33–36]. CAMs are cell surface glycoproteins involved in the establishment of normal tissue structure and function, hence contributing to a variety of physiological processes as morphogenesis, embryogenesis, organogenesis, immunological function, wound healing, and inflammation [37]. Cadherins, integrins, selectins, and members of the immunoglobulin superfamily are the four major groups of CAMs mainly involved in transduction signaling, cytoskeletal organization, and gene regulation upon cell-to-cell and cell-to-ECM interactions [38,39]. Hence, alterations in their expression may contribute to peculiar features of neoplastic transformation, including the loss of cellular morphology and tissue architecture [40,41] as well as cell invasion, migration, EMT, trans-endothelial migration, intra- and extra-vasation, tumor angiogenesis, and organ-specific metastasis [42]. In line with our and other previous studies [12,43,44], the present data analysis determined that one of the most GPER-correlated genes belonging to the CAMs pathway is the microvessel density marker CD34. As further pro-tumorigenic GPER-associated genes belonging to the CAMs pathway, CDH5, CLDN-5, ESAM, CADM3, JAM2, and JAM3 were also identified. In particular, CDH5, CLDN-5, and ESAM were indicated as relevant players in BC progression and recurrence [45–48]. As it concerns CADM3, JAM2, and JAM3, their role in breast malignancies has not yet been elucidated; however, several studies revealed their pro-tumorigenic role in diverse types of malignancy [49–52].

Focal adhesions are protein complexes that connect the cell cytoskeleton to the ECM and then act as scaffolds in outside-in transduction signaling [53–55]. In particular, the FAs-mediated intracellular pathways cooperate with receptor tyrosine kinases toward the regulation of cell shape, polarity, adhesion, migration, differentiation, survival, and proliferation [56]. As it concerns cancer development, an altered expression and function of both ECM and FAs has been shown to be crucial for the dissemination of breast tumor cells and therefore for the acquisition of malignant features [9,54,57–60]. Among the GPER-associated genes belonging to the ECM-receptor interaction and the FA pathway, we found COL6A1, COL6A2, and LAMB2 in particular. An increased collagen deposition was shown to exert a fundamental role within the tumor microenvironment toward cancer growth and escape [61,62], and specifically COL6A1 was involved in both cell proliferation and metastasis of diverse malignancies as BC [63–67]. Likewise, laminins including LAMB2 have been involved in the maintenance and the regulation of cell polarity, anchorage-independent growth, migration and invasion, EMT activity, metastasis, resistance to anoikis, and a poor outcome in BC [68–75]. Further extending our previous findings [43], the present analysis showed a positive correlation between GPER and IGF1 expression in ER-negative BC patients. These data fit well with the capability of IGF1 to regulate GPER expression
Cell growth toward BC growth [28,43,76]. Moreover, in the current study, PDGFRB was demonstrated as an additional FA gene associated with GPER. Although the role of the PDGFB/PDGFRB axis in BC progression is still a subject of debate, PDGFRB overexpression was correlated with the acquisition of vascular-like functional properties of TNBC, suggesting its involvement in tumor aggressiveness [77,78]. From our correlation and pathway analysis, two members of the ECM-receptor interaction and the FA pathway, named PGF and VWF, which were indicated as prognostic markers in BC [79,80], appeared to be associated with GPER.

The correlation between GPER expression and clinicopathological determinants of BC progression, including survival, tumor size, number of positive lymph nodes, and vascular invasion, still remains to be understood [11,12,31,32,81–83]. In this regard, we ascertained that a high expression of GPER correlates with a short DFI in the aggressive BC lacking ER, hence corroborating our findings on the gene expression profile associated with GPER in this subgroup of patients.

To date, controversial findings on the prognostic role of GPER in BC have been reported. For instance, a recent survival analysis demonstrated an association of high expression of GPER with low overall survival of BC patients [81]. In addition, the expression of GPER was indicated as an independent unfavorable factor for relapse-free survival in BC patients treated with tamoxifen [83]. Accordingly, the involvement of GPER in the resistance to tamoxifen was suggested in previous studies [84,85]. Moreover, immunohistochemical investigations associated the lack of GPER in the plasma membrane with an improved long-term prognosis of tamoxifen-treated patients [86]. Overall, these findings point to the need for a better understanding of the role exerted by GPER in breast tumors.

5. Conclusions and Future Perspectives

In the present study, we found a correlation between GPER expression and pro-metastatic genes in ER-negative BC, as assessed querying the TCGA and the METABRIC datasets. In this respect, a deeper understanding of the functional relationships between GPER and these genes would allow the identification of the molecular mechanisms through which GPER may be involved in the aggressive features of breast tumors lacking ER. We also determined that a high expression of GPER correlates with a short DFI in the aforementioned BC subtype; nevertheless, further studies are required to better assess the significance of GPER in breast malignancies characterized by a worse prognosis.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4409/9/3/622/s1, Figure S1: Enrichment plots of KEGG CAMs (A,B) ECM-receptor interaction (C,D) and FA pathway genes (E,F) by GSEA in ER-negative BC patients of TCGA and METABRIC datasets. Enrichment scores (ES) and relative p-values are plotted. A positive enrichment score (ES) indicates an enrichment of the selected gene set at the top of the ranked list. The score is calculated by walking down a list of genes ranked by their correlation with the selected phenotype (high or low GPER levels), increasing a running-sum statistic when a gene in that gene set is encountered (each blue vertical line underneath the enrichment plot) and decreasing it when a gene that isn’t in the gene set is encountered, Table S1: Genes correlated with GPER (n. 277) and shared between TCGA and METABRIC datasets.

Author Contributions: Conceptualization, M.T., E.M.D.F., A.B. and R.L.; data curation, M.T., E.M.D.F., D.C.R., A.B. and A.H.S.; validation, formal analysis and investigation, M.T., E.M.D.F., D.C.R., L.M., M.G.M.; methodology, M.T. and A.H.S.; resources, M.M. and A.H.S.; data curation, writing—original draft preparation, M.T., E.M.D.F., M.G.M., L.M. and R.L.; writing—review and editing, M.M., R.L. and A.H.S.; funding acquisition, M.M. All authors have read and agreed to the published version of the manuscript.

Funding: The research leading to these results has received funding from AIRC under IG 2018-ID. 21322 project—P.I. Marcello Maggiolini, under IG 2019-ID. 23369—P.I. Antonino Belfiore; Ernestina Marianna De Francesca was also supported by AIRC, Start-Up Grant ID. 21651.

Acknowledgments: The Authors acknowledge PON Ricerca e Competitività 2007–2013, Sistema Integrato di Laboratori per L’Ambiente—(SILA) PONa3_00341 for providing lab tools and the special award namely “Department of Excellence 2018-2022” (Italian Law 232/2016) to the Department of Pharmacy, Health and Nutritional Sciences of the University of Calabria (Italy). Damiano Cosimo Rigiracciolo was supported by Italian Minister of University and Research (MIUR, D.D. n. 3407/2018)-PON R&I 2014–2020 “AIM Attrazione e Mobilità Internazionale”.

Conflicts of Interest: The Authors declare that they have no competing interests.
References

1. Bray, F.; Ferlay, J.; Soerjomataram, I.; Siegel, R.L.; Torre, L.A.; Jemal, A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J. Clin. 2018, 68, 394–424. [CrossRef]

2. Eroles, P.; Bosch, A.; Pérez-Fidalgo, J.A.; Lluch, A. Molecular biology in breast cancer: Intrinsic subtypes and signaling pathways. Cancer Treat. Rev. 2012, 38, 698–707. [CrossRef]

3. Huang, B.; Warner, M.; Gustafsson, J.A. Estrogen receptors in breast carcinogenesis and endocrine therapy. Mol. Cell. Endocrinol. 2015, 418, 240–244. [CrossRef]

4. Yaşar, P.; Ayaz, G.; User, S.D.; Güpür, G.; Muyan, M. Molecular mechanism of estrogen-estrogen receptor signaling. Reprod. Med. Biol. 2016, 16, 4–20. [CrossRef]

5. Barton, M.; Filardo, E.J.; Lolait, S.J.; Thomas, P.; Maggiolini, M.; Prossnitz, E.R. Twenty years of the G-protein-coupled estrogen receptor GPER: Historical and personal perspectives. J. Steroid Biochem. Mol. Biol. 2018, 176, 4–15. [CrossRef]

6. Lappano, R.; Pisano, A.; Maggiolini, M. GPER Function in Breast Cancer: An Overview. Front. Endocrinol. (Lausanne) 2014, 5, 66. [CrossRef] [PubMed]

7. Marjón, N.A.; Hu, C.; Hathaway, H.J.; Prossnitz, E.R. G protein-coupled estrogen receptor regulates mammary tumorigenesis and metastasis. Mol. Cancer Res. 2014, 12, 1644–1654. [CrossRef] [PubMed]

8. De Francesco, E.M.; Lappano, R.; Santolla, M.F.; Marsico, S.; Caruso, A.; Maggiolini, M. HIF-1α/GPER signaling mediates the expression of VEGF induced by hypoxia in breast cancer associated fibroblasts (CAFs). Breast Cancer Res. 2013, 15, R64. [CrossRef] [PubMed]

9. Rigiracciolo, D.C.; Santolla, M.F.; Lappano, R.; Vivacqua, A.; Cirillo, F.; Galli, G.R.; Talia, M.; Muglia, L.; Pellegrino, M.; Nohata, N.; et al. Focal adhesion kinase (FAK) activation by estrogens involves GPER in triple-negative breast cancer cells. J. Exp. Clin. Cancer Res. 2019, 38, 58. [CrossRef]

10. Filardo, E.J.; Graeber, C.T.; Quinn, J.A.; Resnick, M.B.; Giri, D.; DeLellis, R.A.; Steinhoff, M.M.; Sabo, E. Distribution of GPR30, a seven membrane-spanning estrogen receptor, in primary breast cancer and its association with clinicopathologic determinants of tumor progression. Clin. Cancer Res. 2006, 12, 6359–6366. [CrossRef]

11. Liu, Q.; Li, J.G.; Zheng, X.Y.; Jin, F.; Dong, H.T. Expression of CD133, PAX2, ESA, and GPR30 in invasive ductal breast carcinomas. Chin. Med. J. (Engl.) 2009, 122, 2763–2769. [PubMed]

12. Martin, S.G.; Lebot, M.N.; Sukkarn, B.; Ball, G.; Green, A.R.; Rakha, E.A.; Ellis, I.O.; Storr, S.J. Low expression of G protein-coupled oestrogen receptor 1 (GPER) is associated with adverse survival of breast cancer patients. Oncotarget 2018, 9, 25946–25956. [CrossRef] [PubMed]

13. Gao, J.; Aksoy, B.A.; Dogrusoz, U.; Dresdner, G.; Gross, B.; Sumer, S.O.; Sun, Y.; Jacobsen, A.; Sinha, R.; Larsson, E.; et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci. Signal. 2013, 6, pl1. [CrossRef] [PubMed]

14. Ho, D.W.; Kai, A.K.; Ng, I.O. TCGA whole-transcriptome sequencing data reveals significantly dysregulated genes and signaling pathways in hepatocellular carcinoma. Front. Med. 2015, 9, 322–330. [CrossRef] [PubMed]

15. Stein, L.D.; Knoppers, B.M.; Campbell, P.; Getz, G.; Korbel, J.O. Data analysis: Create a cloud commons. Nature 2015, 523, 149–151. [CrossRef] [PubMed]

16. Ciriello, G.; Gatza, M.L.; Beck, A.H.; Wilkerson, M.D.; Rheik, S.K.; Pastore, A.; Zhang, H.; McLellan, M.; Yau, C.; Kandoth, C.; et al. Comprehensive Molecular Portraits of Invasive Lobular Breast Cancer. Cell 2015, 163, 506–519. [CrossRef] [PubMed]

17. Curtis, C.; Shah, S.P.; Chin, S.F.; Turashvili, G.; Rueda, O.M.; Dunning, M.J.; Speed, D.; Lynch, A.G.; Samarakawa, S.; Yuan, Y.; et al. The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. Nature 2012, 486, 346–352. [CrossRef]

18. Akbani, R.; Ng, P.K.; Werner, H.M.; Shahmoradgoli, M.; Zhang, F.; Ju, Z.; Liu, W.; Yang, J.Y.; Yoshihara, K.; Li, J.; et al. A pan-cancer proteomic perspective on The Cancer Genome Atlas. Nat. Commun. 2014, 5, 3887. [CrossRef]

19. Filardo, E.J. A role for G-protein coupled estrogen receptor (GPER) in estrogen-induced carcinogenesis: Dysregulated glandular homeostasis, survival and metastasis. J. Steroid Biochem. Mol. Biol. 2018, 176, 38–48. [CrossRef]
20. Huang, d.W.; Sherman, B.T.; Lempicki, R.A. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat. Protoc. 2009, 4, 44–57. [CrossRef]

21. Pearce, D.A.; Nirmal, A.J.; Freeman, T.C.; Sims, A.H. Continuous Biomarker Assessment by Exhaustive Survival Analysis. bioRxiv 2018. [CrossRef]

22. Santolla, M.F.; Vivacqua, A.; Lappano, R.; Rigiracciolo, D.C.; Cirillo, F.; Galli, G.R.; Talia, M.; Brunetti, G.; Miglietta, A.M.; Belfiore, A.; et al. GPER Mediates a Feedforward FGF2/FGFR1 Paracrine Activation Coupling CAFs to Cancer Cells toward Breast Tumor Progression. Cells 2018, 8, 223. [CrossRef] [PubMed]

23. De Francesco, E.M.; Maggiolini, M.; Musti, A.M. Crosstalk between Notch, HIF-1α and GPER in Breast Cancer EMT. Int. J. Mol. Sci. 2018, 19, 2011. [CrossRef] [PubMed]

24. Lappano, R.; Mallet, C.; Rizzuti, B.; Grande, F.; Galli, G.R.; Byrne, I.; Boudieu, L.; Eschalier, A.; Jacquot, Y.; et al. The Peptide ERα17p is a GPER Inverse Agonist that Exerts Antiproliferative Effects in Breast Cancer Cells. Cells 2019, 8, 590. [CrossRef] [PubMed]

25. Arias-Pulido, H.; Royce, M.; Gong, Y.; Joste, N.; Lomo, L.; Lee, S.J.; Chaher, N.; Verschraegen, C.; Lara, J.; Prossnitz, E.R.; et al. GPR30 and estrogen receptor expression: New insights into hormone dependence of inflammatory breast cancer. Breast Cancer Res. Treat. 2010, 123, 51–58. [CrossRef]

26. Woodfield, G.W.; Horan, A.D.; Chen, Y.; Weigel, R.J. TFAP2C controls hormone response in breast cancer cells through multiple pathways of estrogen signaling. Cancer Res. 2007, 67, 8439–8443. [CrossRef]

27. Albanito, L.; Lappano, R.; Madeo, A.; Chimento, A.; Prossnitz, E.R.; Cappello, A.R.; Dolce, V.; Abonante, S.; Pezzi, V.; Maggiolini, M. Effects of atrazine on estrogen receptor α- and G protein-coupled receptor 30-mediated signaling and proliferation in cancer cells and cancer-associated fibroblasts. Environ. Health Perspect. 2015, 123, 493–499. [CrossRef]

28. De Marco, P.; Bartella, V.; Vivacqua, A.; Lappano, R.; Santolla, M.F.; Morcavallo, A.; Pezzi, V.; Belfiore, A.; Maggiolini, M. Insulin-like growth factor-I regulates GPER expression and function in cancer cells. Oncogene 2013, 32, 678–688. [CrossRef]

29. Vivacqua, A.; Lappano, R.; De Marco, P.; Sisci, D.; Aquila, S.; De Amicis, F.; Fuqua, S.A.; Andò, S.; Maggiolini, M. G protein-coupled receptor 30 expression is up-regulated by EGF and TGF alpha in estrogen receptor alpha-positive cancer cells. Mol. Endocrinol. 2009, 23, 1815–1826. [CrossRef]

30. Albanito, L.; Madeo, A.; Lappano, R.; Vagacqua, A.; Rago, V.; Carpino, A.; Oprea, T.I.; Prossnitz, E.R.; Musti, A.M.; Andò, S.; et al. 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 Res. 2007, 67, 1859–1866. [CrossRef]

31. Aiad, H.A.; Wahed, M.M.; Asaad, N.Y.; El-Tahmody, M.; Elhosary, E. Immunohistochemical expression of GPR30 in breast carcinoma of Egyptian patients: An association with immunohistochemical subtypes. APIMS 2014, 122, 976–984. [CrossRef] [PubMed]

32. Broselid, S.; Cheng, B.; Sjöström, M.; Lövgren, K.; Klug-De Santiago, H.L.; Belting, M.; Jirström, K.; Malmström, P.; Olde, B.; Bendahl, P.O.; et al. G protein-coupled estrogen receptor is apoptotic and correlates with increased distant disease-free survival of estrogen receptor-positive breast cancer patients. Clin. Cancer Res. 2013, 19, 1681–1692. [CrossRef] [PubMed]

33. Deng, Q.; Jiang, G.; Wu, Y.; Li, J.; Liang, W.; Chen, L.; Su, Q.; Li, W.; Du, J.; Wong, C.K.C.; et al. GPER/Hippo-YAP signal is involved in Bisphenol S induced migration of triple negative breast cancer (TNBC) cells. J. Hazard. Mater. 2018, 355, 1–9. [CrossRef] [PubMed]

34. Castillo Sanchez, R.; Gomez, R.; Perez Salazar, E. Bisphenol A Induces Migration through a GPER-, FAK-, Src-, and ERK2-Dependent Pathway in MDA-MB-231 Breast Cancer Cells. Chem. Res. Toxicol. 2016, 29, 285–295. [CrossRef]

35. Zhou, X.; Wang, S.; Wang, Z.; Feng, X.; Liu, P.; Lv, X.B.; Li, F.; Yu, F.X.; Sun, Y.; Yuan, H.; et al. Estrogen regulates Hippo signaling via GPER in breast cancer. J. Clin. Investig. 2015, 125, 2123–2135. [CrossRef]

36. Filardo, E.J.; Quinn, J.A.; Sabo, E. Association of the membrane estrogen receptor, GPR30, with breast tumor metastasis and transactivation of the epidermal growth factor receptor. Steroids 2008, 73, 870–873. [CrossRef]

37. Makrilia, N.; Kollias, A.; Manolopoulos, L.; Syrigos, K. Cell adhesion molecules: Role and clinical significance in cancer. Cancer Invest. 2009, 27, 1023–1037. [CrossRef]

38. Theocaris, A.D.; Skandalis, S.S.; Gialelei, C.; Karamanos, N.K. Extracellular matrix structure. Adv. Drug Deliv. Rev. 2016, 97, 4–27. [CrossRef]
39. Humphrey, J.D.; Dufresne, E.R.; Schwartz, M.A. Mechanotransduction and extracellular matrix homeostasis. *Nat. Rev. Mol. Cell Biol.* 2014, 15, 802–812. [CrossRef]

40. He, X.; Lee, B.; Jiang, Y. Cell-ECM Interactions in Tumor Invasion. *Adv. Exp. Med. Biol.* 2016, 936, 73–91.

41. Bonnans, C.; Chou, J.; Werb, Z. Remodelling the extracellular matrix in development and disease. *Nat. Rev. Mol. Cell Biol.* 2014, 15, 786–801. [CrossRef] [PubMed]

42. Li, D.M.; Feng, Y.M. Signaling mechanism of cell adhesion molecules in breast cancer metastasis: Potential therapeutic targets. *Breast Cancer Res. Treat.* 2011, 128, 7–21. [CrossRef] [PubMed]

43. De Francesco, E.M.; Sims, A.H.; Maggiolini, M.; Sotgia, F.; Lisanti, M.P.; Clarke, R.B. GPER mediates the angiocrine actions induced by IGF1 through the HIF-1α/VEGF pathway in the breast tumor microenvironment. *Breast Cancer Res. Treat.* 2017, 19, 129. [CrossRef] [PubMed]

44. De Francesco, E.M.; Pellegrino, M.; Santolla, M.F.; Lappano, R.; Ricchio, E.; Abonante, S.; Maggiolini, M. GPER mediates activation of HIF1α/VEGF signaling by estrogens. *Cancer Res.* 2014, 74, 4053–4064. [CrossRef]

45. Rezaei, M.; Cao, J.; Friedrich, K.; Kemper, B.; Brendel, O.; Grosser, M.; Adrian, M.; Baretton, G.; Breier, G.; Schnittler, H.J. The expression of VE-cadherin in breast cancer cells modulates cell dynamics as a function of tumor differentiation and promotes tumor-endothelial cell interactions. *Histochem. Cell Biol.* 2018, 149, 15–30. [CrossRef]

46. Bartolome, R.A.; Torres, S.; Isern de Val, S.; Escudero-Paniagua, B.; Calviño, E.; Teixidó, J.; Casal, J.I. VE-cadherin RGD motifs promote metastasis and constitute a potential therapeutic target in melanoma and breast cancers. *Oncotarget* 2017, 8, 215–227.

47. Sugimoto, H.; Nagahara, M.; Bae, Y.; Nakagawa, T.; Ishikawa, T.; Sato, T.; Uetake, H.; Eishi, Y.; Sugihara, K. Clinicopathologic relevance of claudin 5 expression in breast cancer. *Am. J. Clin. Pathol.* 2015, 143, 540–546. [CrossRef]

48. Cangara, H.M.; Ishida, T.; Hara, T.; Sun, L.; Toh, R.; Rikitake, Y.; Jiang, L.; Jiang, S.; Hao, K.; Zhang, W. miR-140-5p suppresses retinoblastoma cell proliferation, migration, and invasion by targeting CEMIP and CADM3. *Cell. Mol. Biol.* (Noisy-le-grand) 2018, 64, 42–47. [CrossRef]

49. Miao, X.; Wang, Z.; Yang, Y.; Wang, X.; Jiang, L.; Jiang, S.; Hao, K.; Zhang, W. miR-140-5p suppresses retinoblastoma cell proliferation, migration, and invasion by targeting CEMIP and CADM3. *Cell. Mol. Biol.* (Noisy-le-grand) 2018, 64, 42–47. [CrossRef]

50. Zhao, H.; Yu, H.; Martin, T.A.; Teng, X.; Jiang, W.G. The role of JAM-B in cancer and cancer metastasis (Review). *Oncol. Rep.* 2016, 36, 3–9. [CrossRef]

51. Garrido-Urbani, S.; von Laufen, A.; Stalin, J.; De Grandis, M.; Roapraz, P.; Jemelin, S.; Bardin, F.; Scheib, H.; Aurand-Lions, M.; Imhof, B.A. Junctional adhesion molecule C (JAM-C) dimerization aids cancer cell migration and metastasis. *Biochim. Biophys. Acta Mol. Cell Res.* 2018, 1865, 638–649. [CrossRef] [PubMed]

52. Hao, S.; Yang, Y.; Liu, Y.; Yang, S.; Wang, G.; Xiao, J.; Liu, H. JAM-C promotes lymphangiogenesis and nodal metastasis in non-small cell lung cancer. *Tumour Biol.* 2014, 35, 5675–5687. [CrossRef] [PubMed]

53. Shen, J.; Cao, B.; Wang, Y.; Ma, C.; Zeng, Z.; Liu, L.; Li, X.; Tao, D.; Gong, J.; Xie, D. Hippo component YAP promotes focal adhesion and tumour aggressiveness via transcriptionally activating THBS1/FAK signalling in breast cancer. *J. Exp. Clin. Cancer Res.* 2018, 37, 175. [CrossRef] [PubMed]

54. Nardone, G.; Oliver-De La Cruz, J.; Vrbsky, J.; Martini, C.; Pribyl, J.; Skládal, P.; Pesl, M.; Caluori, G.; Pagliari, S.; Martino, F.; et al. YAP regulates cell mechanics by controlling focal adhesion assembly. *Nat. Commun.* 2017, 8, 15321. [CrossRef]

55. Katoh, K. FAK-Dependent Cell Motility and Cell Elongation. *Cells* 2020, 9, 192. [CrossRef]

56. Stuchbury, B.; Atherton, P.; Tsang, R.; Wang, D.; Ballestrem, C. Distinct focal adhesion protein modules control different aspects of mechanotransduction. *J. Cell Sci.* 2017, 130, 1612–1624. [CrossRef]

57. Di Cara, G.; Marabetti, M.R.; Musso, R.; Riili, I.; Cancemi, P.; Pucci Minafra, I. New Insights into the Occurrence of Matrix Metalloproteases -2 and -9 in a Cohort of Breast Cancer Patients and Proteomic Correlations. *Cells* 2018, 7, 89. [CrossRef]

58. Montagner, M.; Dupont, S. Mechanical Forces as Determinants of Disseminated Metastatic Cell Fate. *Cells* 2020, 9, 250. [CrossRef]

59. Lin, T.; Yang, C.; Cheng, L.; Chang, W.; Lin, Y.; Cheng, H. Fibronectin in Cancer: Friend or Foe. *Cells* 2020, 9, 27. [CrossRef]

60. Raab-Westphal, S.; Marshall, J.F.; Goodman, S.L. Integrins as Therapeutic Targets: Successes and Cancers. *Cancers* 2017, 9, 110. [CrossRef]
61. Karousou, E.; D’Angelo, M.L.; Kouvidi, K.; Vigetti, D.; Viola, M.; Nikitovic, D.; De Luca, G.; Passi, A. Collagen VI and hyaluronan: The common role in breast cancer. *Biomed. Res. Int.* 2014, 2014, 606458. [CrossRef] [PubMed]

62. Cox, T.R.; Erler, J.T. Remodeling and homeostasis of the extracellular matrix: Implications for fibrotic diseases and cancer. *Dis. Model. Mech.* 2011, 4, 165–178. [CrossRef] [PubMed]

63. Owusu-Ansah, K.G.; Song, G.; Chen, R.; Edoo, M.I.A.; Li, J.; Chen, B.; Wu, J.; Zhou, L.; Xie, H.; Jiang, D.; et al. COL6A1 promotes metastasis and predicts poor prognosis in patients with pancreatic cancer. *Int. J. Oncol.* 2019, 55, 391–404. [CrossRef] [PubMed]

64. Panagopoulos, V.; Leach, D.A.; Zinonos, I.; Ponomarev, V.; Ricardi, G.; Liapis, V.; Ingman, W.V.; Anderson, P.; DeNichilo, M.O.; Ekdokioiu, A. Inflammatory peroxidases promote breast cancer progression in mice via regulation of the tumour microenvironment. *Int. J. Oncol.* 2017, 50, 1191–1200. [CrossRef] [PubMed]

65. Hou, T.; Tong, C.; Kazobinka, G.; Zhang, W.; Huang, X.; Huang, Y.; Zhang, Y. Expression of COL6A1 predicts prognosis in cervical cancer patients. *Am. J. Transl. Res.* 2016, 8, 2838–2844. [PubMed]

66. Zhu, Y.P.; Wan, F.N.; Shen, Y.J.; Wang, H.K.; Zhang, G.M.; Ye, D.W. Reactive stroma component COL6A1 is upregulated in castration-resistant prostate cancer and promotes tumor growth. *Oncotarget* 2015, 6, 14488–14496. [CrossRef]

67. Chiu, K.H.; Chang, Y.H.; Wu, Y.S.; Lee, S.H.; Liao, P.C. Quantitative secretome analysis reveals that COL6A1 is a metastasis-associated protein using stacking gel-aided purification combined with iTRAQ labeling. *J. Proteome Res.* 2011, 10, 1110–1125. [CrossRef]

68. Insua-Rodriguez, J.; Oskarsson, T. The extracellular matrix in breast cancer. *Adv. Drug Deliv. Rev.* 2016, 97, 41–55. [CrossRef]

69. Kwon, S.Y.; Chae, S.W.; Wilczynski, S.P.; Arain, A.; Carpenter, P.M. Laminin-332 expression in breast carcinoma, applied immunohistochemistry & molecular morphology. *Appl. Immunohistochem. Mol. Morphol.* 2012, 20, 159–164.

70. Gordon-Weeks, A.; Lim, S.Y.; Yuzhalin, A.; Lucotti, S.; Vermeer, J.A.F.; Jones, K.; Chen, J.; Muschel, R.J. Tumour-Derived Laminin α5 (LAMA5) Promotes Colorectal Liver Metastasis Growth, Branching Angiogenesis and Notch Pathway Inhibition. *Cancers* 2019, 11, 630. [CrossRef]

71. Quo, X.; Tan, H.; Fu, D.; Zhu, Y.; Zhang, J. Laminin is over expressed in breast cancer and facilitate cancer cell metastasis. *J. Cancer Res. Ther.* 2018, 14, S1170–S1172. [PubMed]

72. Kusuma, N.; Denoyer, D.; Eble, J.A.; Redvers, R.P.; Parker, B.S.; Pelzer, R.; Anderson, R.L.; Pouliot, N. Integrin-dependent response to laminin-511 regulates breast tumor cell invasion and metastasis. *Int. J. Cancer* 2012, 130, 555–566. [CrossRef] [PubMed]

73. Procarci, P.; Moscheni, C.; Sartori, P.; Sommariva, M.; Gagliano, N. Tumor-Stroma Cross-Talk in Human Pancreatic Ductal Adenocarcinoma: A Focus on the Effect of the Extracellular Matrix on Tumor Cell Phenotype and Invasive Potential. *Cells* 2018, 7, 158. [CrossRef]

74. Kim, B.G.; Gao, M.Q.; Choi, Y.P.; Kang, S.; Park, H.R.; Kang, K.S.; Cho, N.H. Invasive breast cancer induces laminin-332 upregulation and integrin βα4 neoexpression in myofibroblasts to confer an anoikis-resistant phenotype during tissue remodeling. *Breast Cancer Res. Treat.* 2012, 14, R88. [CrossRef] [PubMed]

75. Agboola, A.O.; Ebili, H.O.; Iyawe, V.O.; Banjo, A.A.; Salami, B.S.; Rakha, E.A.; Nolan, C.; Ellis, I.O.; Green, A.R. Tumour cell membrane laminin expression is associated with basal-like phenotype and poor survival in Nigerian breast cancer. *Malays. J. Pathol.* 2016, 38, 83–92. [PubMed]

76. De Marco, P.; Romeo, E.; Vivaqua, A.; Malaguarnera, R.; Abonante, S.; Romeo, F.; Pezzi, V.; Belfiore, A.; Maggiolini, M. GPER1 is regulated by insulin in cancer cells and cancer-associated fibroblasts. *Endocr. Relat. Cancer* 2014, 21, 739–753. [CrossRef] [PubMed]

77. Camorani, S.; Hill, B.S.; Collina, F.; Gargiulo, S.; Napolitano, M.; Cantile, M.; Di Bonito, M.; Botti, G.; Fedele, M.; Zannetti, A.; et al. Targeted imaging and inhibition of triple-negative breast cancer metastases by a PDGFβR aptamer. *Theranostics* 2018, 8, 5178–5199. [CrossRef]

78. Jansson, S.; Aaltoenen, K.; Bendahl, P.O.; Falck, A.K.; Karlsson, M.; Pietras, K.; Rydén, L. The PDGF pathway in breast cancer is linked to tumour aggressiveness, triple-negative subtype and early recurrence. *Breast Cancer Res. Treat.* 2018, 169, 231–241. [CrossRef]

79. Maee, E.; Olsen, D.A.; Steffensen, K.D.; Jakobsen, E.H.; Brandslund, I.; Sørensen, F.B.; Jakobsen, A. Prognostic impact of placenta growth factor and vascular endothelial growth factor A in patients with breast cancer. *Breast Cancer Res. Treat.* 2012, 133, 257–265. [CrossRef]
80. Lehrer, S.; Green, S.; Dembitzer, F.R.; Rheinstein, P.H.; Rosenzweig, K.E. Increased RNA Expression of von Willebrand Factor Gene Is Associated With Infiltrating Lobular Breast Cancer and Normal PAM50 Subtype. *Cancer Genom. Proteom.* 2019, 16, 147–153. [CrossRef]

81. Ye, S.; Xu, Y.; Li, J.; Zheng, S.; Sun, P.; Wang, T. Prognostic role of GPER/Ezrin in triple-negative breast cancer is associated with menopausal status. *Endocr. Connect.* 2019, 8, 661–671. [CrossRef] [PubMed]

82. Ignatov, T.; Weißenborn, C.; Poehlmann, A.; Lemke, A.; Semczuk, A.; Roessner, A.; Costa, S.D.; Kalinski, T.; Ignatov, A. GPER-1 expression decreases during breast cancer tumorigenesis. *Cancer Invest.* 2013, 31, 309–315. [CrossRef] [PubMed]

83. Ignatov, A.; Ignatov, T.; Weissenborn, C.; Eggemann, H.; Bischoff, J.; Semczuk, A.; Roessner, A.; Costa, S.D.; Kalinski, T. G-protein-coupled estrogen receptor GPR30 and tamoxifen resistance in breast cancer. *Breast Cancer Res. Treat.* 2011, 128, 457–466. [CrossRef] [PubMed]

84. Catalano, S.; Giordano, C.; Panza, S.; Chemi, F.; Bonofiglio, D.; Lanzino, M.; Rizza, P.; Romeo, F.; Fuqua, S.A.; Maggiolini, M.; et al. Tamoxifen through GPER upregulates aromatase expression: A novel mechanism sustaining tamoxifen-resistant breast cancer cell growth. *Breast Cancer Res. Treat.* 2014, 146, 273–285. [CrossRef] [PubMed]

85. Ignatov, A.; Ignatov, T.; Roessner, A.; Costa, S.D.; Kalinski, T. Role of GPR30 in the mechanisms of tamoxifen resistance in breast cancer MCF-7 cells. *Breast Cancer Res. Treat.* 2010, 123, 87–96. [CrossRef]

86. Sjöström, M.; Hartman, L.; Grabau, D.; Fornander, T.; Malmström, P.; Nordenskjöld, B.; Sgroi, D.C.; Skoog, L.; Stål, O.; Leeb-Lundberg, L.M.; et al. Lack of G protein-coupled estrogen receptor (GPER) in the plasma membrane is associated with excellent long-term prognosis in breast cancer. *Breast Cancer Res. Treat.* 2014, 145, 61–71. [CrossRef]

© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).