Systematic in silico analysis suggests a protective role for progesterone receptor in pancreatic adenocarcinoma

Gulbahar Boyuk Ozcan[1] and Burcu Biterge Sut[2*]

[1]Ankara Medipol University, Faculty of Medicine, Department of Physiology, Ankara, Turkey. Orcid ID: https://orcid.org/0000-0002-3453-2967

[2*]Istanbul Technical University, Faculty of Science and Letters, Department of Molecular Biology and Genetics, İstanbul, Turkey. Orcid ID: https://orcid.org/0000-0001-5756-5756

***Corresponding author:** Assoc. Prof. Dr. Burcu Biterge Sut

Abstract

Pancreatic adenocarcinoma (PAAD) is a highly lethal cancer, which is characterized by its aggressiveness and late diagnosis. Progesterone receptor (PGR) has been associated with various malignancies in literature but its role in PAAD remains poorly understood. In this study, we investigated the role of progesterone receptor in PAAD using *in silico* analyses. For this purpose, we examined PGR mutations and expression levels in relation to genomic alteration frequencies, tumor infiltration and disease prognosis in TCGA PAAD datasets. Our findings showed that PGR is expressed in low levels in tumor samples and is highly methylated at its promoter. The low expression levels of PGR correlated with significantly higher alteration event frequencies, increased genomic instability and poor prognosis. Furthermore, we identified missense substitutions of R319C, R623H and R869H in the PGR gene, which resulted in decreased protein stability and were predicted to be pathogenic. Low levels of PGR expression also correlated with lower levels of tumor infiltrating CD8+ T cells, macrophages, neutrophils, and dendritic cells. Overall, our results suggest a protective function for PGR in pancreatic adenocarcinoma, which could indicate PGR as a potential prognostic biomarker for PAAD.

Keywords: Progesterone receptor, PGR, gene expression, pancreatic adenocarcinoma, PAAD, cancer.

1. Introduction

The word "cancer" refers to a wide range of illnesses that are marked by unchecked cell development and the ability to spread (metastasize) to different areas of the body (Teunissen et al., 2007). The exocrine glands of the pancreas, an organ vital for digestion and blood sugar management, are the site of formation of pancreatic cancer. Pancreatic adenocarcinoma (PAAD) is the most prevalent kind of pancreatic cancer and is characterized by its aggressiveness and frequently late diagnosis. Its propensity to spread quickly and resistance to numerous treatments

usually mean a dismal prognosis. The symptoms of PAAD such as upper abdomen pain, jaundice (skin and eyes turning yellow), weight loss, back discomfort, stool color or consistency changes (Shi and Gao, 2022) do not usually become apparent until the cancer has progressed. Smoking, obesity, family history, and specific genetic disorders are risk factors for PAAD. Current treatment options for PAAD include surgery, chemotherapy and adjuvant therapy depending on the stage and the situation. Regrettably, there are less curative therapeutic options available as PAAD frequently progresses before symptoms manifest. Ongoing research, however, seeks to enhance early detection and create more potent treatments (Thomas et al., 2010).

Progesterone receptor (PGR) belongs to the ligand-activated steroid hormone receptor superfamily, and is essential for controlling several physiological functions, such as cell division, proliferation, and apoptosis (Escriva, Bertrand and Laudet, 2004). Members of the steroid hormone receptor family can act as transcriptional activators or repressors in response to hormonal induction (Edwards, 2000). The interaction between PGR and progesterone is involved in the regulation of gene expression, which in turn affects cell division and growth (Mulac-Jericevic and Conneely, 2004). This way, PGR plays key roles in several hormone-related malignancies and research has shown that PGR activation may result in decreased tumor growth in breast (Carroll et al., 2017), colon (Alrushaid et al., 2023) ovarian and endometrial cancers (van Kruchten et al., 2015). Similarly, PGR status is frequently utilized to inform therapy choices in breast cancer as in patients with ER/PR-positive breast and endometrial cancer, hormone treatment inhibits the growth of the tumor by targeting PGR and preventing its interaction with progesterone (Mileshkin et al., 2016).

PGR's function in other forms of cancer, such as pancreatic adenocarcinoma (PAAD), is less clear. There are only a limited number of studies in the literature investigating the role of PGR in pancreatic cancer and these studies collectively suggest a protective function for PGR signaling in pancreatic carcinogenesis, where it is linked with increased cell cycle arrest, decreased epithelial-to-mesenchymal transition (EMT), and reduced inflammation (Goncharov et al., 2017), as well as induction of apoptosis (Abe, Yamashita and Ogawa, 2000) and growth inhibition in human pancreatic tumor cell lines (Benz, Hollander and Miller, 1986). Furthermore, PGR expression was reported to be significantly correlated with the absence of metastases and the lack of tumor invasion in pancreatic endocrine tumors (Viale et al., 1992). In addition, we previously showed that pancreatic islet cells respond to progesterone secretion from luteal cells when co-cultured in vitro (Boyuk, Yigit and Aydogan, 2018). Despite these efforts, a complete picture of the molecular mechanisms of PGR operation in pancreatic cancer is yet to be fully understood. Therefore, this study aims to gain a better understanding of PGR's significance in different cancer types and its potential as a therapeutic target or prognostic marker via investigation of PGR expression profiles in pancreatic adenocarcinoma patients in relation to survival, genomic alterations, tumor infiltration and activation of signaling pathways using in silico analysis tools.

2. Material and methods

2.1 Mutation analysis

Complete list of somatic mutations of the PGR gene (COSMIC gene ID: COSG106896) encoding human PGR protein were retrieved from the Catalogue of Somatic Mutations in Cancer (COSMIC)

database v100 (https://cancer.sanger.ac.uk/cosmic) and cBio Cancer Genomics Portal (http://cbioportal.org), which is a publicly available data analysis tool based on large-scale genomics projects (Cerami et al., 2012).

2.2 Prediction of protein stability and pathogenicity

Pathogenicity scores were predicted using dbNSFP, which is a database developed for functional prediction and annotation of all potential non-synonymous single-nucleotide variants (nsSNVs) in the human genome (Liu et al. 2020). The analysis returns a MetaRNN score, which can range from 0.0 to 1.0 and higher MetaRNN scores indicate higher probability of pathogenicity.

Prediction of protein stability in relation to PGR mutations was performed via MUpro using full-length human PGR amino acid sequence (UniProt ID: P06401) (http://mupro.proteomics.ics.uci.edu) (Cheng et al. 2006). Structural alterations due to identified pathogenic mutations were determined via HOPE server (https://www3.cmbi.umcn.nl/hope), which also provided 3D images of the pathogenic PGR mutations based on protein models created by AlphaFold-2 (Venselaar et al. 2010, Varadi et al. 2024).

2.3 Expression analysis

The mRNA expression levels of PGR in TCGA tumor vs. normal tissue samples, the expression of PGR based on TP53 mutation status and the promoter methylation levels of PGR in PAAD were retrieved from UALCAN (The University of Alabama at Birmingham cancer data analysis portal),

which provides comprehensive analysis of and access to publicly available cancer OMICS data (Chandrashekar et al., 2022). PGR protein expression was analyzed in immunohistochemistry images of pancreas adenocarcinoma tissue samples stained using anti-PGR antibody (HPA017176) available on the Human Protein Atlas (https://www.proteinatlas.org) (Uhlen et al. 2015).

2.4 Survival analysis

Kaplan-Meier survival analysis was performed on KM Plotter (https://kmplot.com/analysis/) (Győrffy, 2024). The effect of PGR mRNA expression levels on overall survival and disease-free survival was assessed using mRNA gene chip data of 1237 and 278 pancreatic cancer patients, respectively for each analysis, using default settings. Analysis of overall survival in TP-mutated and nonmutated groups was performed using cBioPortal (Cerami et al., 2012).

2.5 Determination of genomic alteration frequencies

First, two subgroups of patients were generated according to their relative PGR expression levels in comparison to the mean value for PGR expression for the whole patient cohort. Z-score of zero indicates the mean expression level across all samples; therefore, patients with PGR expression above the mean value (z-score>0) were classified as the PGR high group, while the patients with PGR expression below the mean value (z-score<0) were classified as the PGR low group. By using the Comparison/Survival segment on cBioPortal, mRNA expression levels of the genes of interest were comparatively analyzed between PGR low/high groups.

Genomic alterations module of cBioPortal calculates the frequency of mutations (missense, inframe, truncating, deletion, and insertion), structural variants, fusions and copy number alterations (deletions and amplifications) in PGR high/low patients and depicts the top 10 genes with highest frequency in these groups as a bar chart, which we used for the comparative analysis of genomic alteration frequencies.

2.6 Analysis of immune infiltration

The correlation between the mRNA expression level of PGR and tumor infiltration levels of immune cell subtypes (B cells, CD4+ T cells, CD8+ T cells, macrophages, neutrophils, and dendritic cells) in pancreatic adenocarcinoma was analyzed using TIMER: Tumor IMmune Estimation Resource (http://timer.cistrome.org/), which generated scatterplots of tumor purity corrected expression levels in comparison to immune cell infiltration levels (Li et al., 2020).

2.7 Statistical analysis

All p-values were automatically calculated by the respective tool for statistical analysis and p-values below 0.05 (%5) were considered significant. TIMER calculates partial Spearman's rho values (correlation coefficients) for statistical analysis and correlation coefficients above 0.3 were considered significant. KM Plotter offers statistical analysis based on methods including Cox proportional hazards regression and the computation of the False Discovery Rate.

3. Results

First, we examined the mRNA expression levels of PGR in TCGA tumor vs. normal tissue samples available in UALCAN database and showed that PGR expression is lower in the PAAD tumors in comparison to the normal pancreatic tissue samples (Figure 1.a). We analyzed 10 immunohistochemistry images obtained from the Human Protein Atlas, which had high, medium or low levels of PGR staining or no detection (Figure 1.b). The majority of pancreas adenocarcinoma tissue samples had medium or low PGR-antibody staining levels and the subcellular distribution PGR staining was confined to cytoplasmic and membranous compartments (Figure 1.c). We also found that the levels of promoter methylation, which is indicative of gene expression repression, was significantly higher ($p<0.05$) in the PAAD tumors in comparison to the normal pancreatic tissue (Figure 1.d). In line with the reduced expression of PGR in tumor tissue, Kaplan-Meier survival analysis indicated that PAAD patients with elevated expression of PGR had a survival advantage. Higher levels of PGR were correlated with a higher probability of both overall (not significant, $p>0.05$) and disease-free survival (significant, $p < 0.001$), suggesting better prognosis (Figure 1.e-f).

Among the TCGA pancreatic adenocarcinoma patient dataset, 168 patients had mRNA expression data, while the number of patients with protein expression data was 109. In all patient samples that had data for both mRNA and protein, PGR expression was detected at varying levels (Figure 2.a). To better dissect the effect of PGR expression in PAAD, we classified the patients into two subgroups according to their PGR expression levels. We used z-scores to compare PGR mRNA expression levels between patient samples. Cancer is a disease of recurrent genomic alterations in tumor suppressors and oncogenes. Therefore, we analyzed the genes that most frequently undergo genomic alterations such as mutations, fusions and copy number alterations within the study group, in order to identify molecular differences that could be influenced or accompanied by varying PGR

expression levels in PAAD. Top 10 most frequently altered genes were identified as KRAS, CDKN2A/B and their variants, TP53, SMAD-4, MTAP and IFNA1 (Figure 2.b). Among these genes; KRAS, CDKN2A and SMAD-4 had significantly higher alteration event frequencies in the PGR low group. Likewise, the rest of the genes had the same tendency while it was not statistically significant, which collectively suggests increased genomic instability in the PGR low group. Interestingly, PGR expression was significantly lower in the TP53-mutant TCGA samples when compared with the nonmutant tissue samples (Figure 2.c). In parallel to the proposed survival advantage of higher PGR expression, patients with nonmutant-TP53 had a significantly longer overall survival ($p<0.05$), indicating better prognosis (Figure 2.d). We also found that the expression levels of KRAS, TP53, CDKN2A and SMAD-4 genes also varied between PGR low/high groups (Figure 2.e). TP53 expression was significantly higher ($p<0.05$) and the SMAD4 expression was significantly lower ($p<0.001$) in the PGR low group. Furthermore, we analyzed the top 20 genes with the highest expression in each group to assess whether PGR low/high groups exhibit differential gene expression profiles at the transcriptome level, which revealed no overlap between groups (Table 1 and Table 2).

Since mutations are important component of genomic alterations, which occur frequently in cancer, we next set out to analyze PGR variants in PAAD. COSMIC database analysis identified a total of 588 somatic PGR missense substitutions in several cancer types, especially arising from primary tissues of large intestine, skin, lung and stomach. Of these, the substitutions of D225G, R242Q, A247T and Y374S were specific to the neoplasms of pancreas. On the other hand, S344T, R623H, R788Q and R869H mutations were found in PAAD, as well as a variety of other cancer types. Additionally, we identified A218V, R319C and Q556H mutations in PGR via cBioPortal analysis (Table 3). All of these mutations were predicted to result in a decrease in protein stability

at varying levels. dbNSFP analysis predicted R319C, R623H and R869H mutations as pathogenic as MetaRNN scores closer to 1.0 indicates pathogenicity. All mutations were located at functionally important domains such as progesterone receptor domain, C4 type Zinc finger domain, which is generally found in DNA-binding regions of some well-characterized families of nuclear receptors, and the ligand-binding domain of nuclear hormone receptor (Figure 3a). As missense substitutions often introduce differences in size, charge, and hydrophobicity, PGR mutations could cause structural alterations and disturb the functions of these domains. In particular, the potentially pathogenic mutations of R319C, R623H and R869H result in the loss of the positively charged side chain of arginine and at the same time give rise to smaller residues (Figure 3.b). The wild-type arginine at position 623 is located within a Zinc finger domain and is involved in a metal-ion contact. It also forms a hydrogen bond with cysteine at position 169. Similarly, arginine at position 869 forms hydrogen bonds with leucine 811, cysteine 812 and glutamate 817. In addition, it forms a salt bridge with glutamate 817. Therefore, the size differences introduced by R623H and R869H mutations most likely result in the new residue to be in an incorrect position to make these hydrogen bonds or salt bridges as the original wild-type residues.

Lastly, after establishing that there are significant molecular differences between the patient subgroups with high/low expression levels of PGR, we set out to evaluate their potential effects on clinical outcomes. Towards this aim, we investigated the immune infiltration levels in pancreatic cancer in relation to PGR expression, which is a critical parameter of cancerous growth, capability of migration and invasion. We found that the infiltration levels of CD8+ T cells, macrophages, neutrophils and dendritic cells showed significant correlation with PGR expression (correlation coefficient>0.3 and $p<0.001$) (Figure 4).

4. Discussion

Steroid hormones such as estrogen, progesterone and testosterone are typically considered as sex hormones and evaluated in the context of sexual development and fertility. The interaction between progesterone and its receptor is essential for the regulation of female reproductive function and PGR is highly expressed in the related tissues such as uterus, ovary and mammary gland (Graham and Clarke, 1997). It is now well established that PGR expression in tumors of these tissues affects disease prognosis and response to treatment (van Kruchten et al., 2015; Carroll et al., 2017). However, steroid hormone receptors are present in a variety of tissues including but not limited to central nervous system, cardiovascular system and the bone (Scarpin et al., 2009). Therefore, in this study, we analyzed PGR mutations and expression profiles in TCGA datasets obtained from pancreatic adenocarcinoma patients in relation to genomic alterations, tumor infiltration and survival.

We detected PGR expression in all patients within the PAAD dataset, though at varying levels, which is in line with previous studies in the literature reporting PGR expression in pancreatic tissue and tumors (Doglioni et al., 1990; Targarona et al., 1991), as well as pancreatic carcinoma cell lines (Selvan, Metzgar and Petrow, 1992; Abe, Yamashita and Ogawa, 2000). PGR expression was low both in TCGA tumor samples and the PGR antibody-stained pancreas adenocarcinoma tissue samples, which was accompanied by the higher DNA methylation at the PGR promoter. The low-level expression of PGR in tumor samples was correlated with shorter survival periods, as well as increased genomic instability evident by the higher alteration event frequencies of the four main

drivers of pancreatic cancer, namely KRAS, p53, CDKN2A and SMAD-4 (Jones et al., 2008; Hayashi, Hong and Iacobuzio-Donahue, 2021). Furthermore, patients with TP53-mutations had lower PGR expression in comparison to the patients with wild-type TP53, which significantly affected overall survival as well. Both shorter survival periods and increased genomic instability are associated with poor prognosis in cancer (Yao and Dai, 2014), suggesting a protective role for higher PGR expression in PAAD. KRAS is an oncogene and is often transformed by an activating mutation during carcinogenesis that maintain a continuous KRAS signaling for cellular growth and survival (Jaffee et al., 2002; Collins et al., 2012). CDKN2A is one of the most important and frequently inactivated tumor suppressor genes in pancreatic cancer. It has been shown that its cooperation with the oncogene KRAS favors the development of pancreatic ductal adenocarcinoma (Maitra, Kern and Hruban, 2006). Likewise, p53 is highly mutated and inactivated in cancers including PAAD, which results in survival and growth advantage for the cancerous cell (Redston et al., 1994). SMAD-4, a key component of the TGF-β signaling pathway, is inactivated in more than 50% of pancreatic cancer patients (Siegel and Massagué, 2003). MTAP was identified as another frequently altered gene within the PGR low group, which is considered as a tumor suppressor gene that is frequently co-deleted with CDKN2A in several cancer types, including pancreatic carcinoma (Bertino et al., 2011).

Carcinogenesis is often driven by mutations in genes critical for the maintenance of cell function and cellular identity. Despite the accumulating evidence for the importance of PGR expression in several types of cancer, PGR mutations are largely overlooked. There is only one study, which determined the frequency and prognostic significance of PGR mutations in metastatic breast cancer patients, where they identified 71 protein-coding PGR variants including S344T and R623H mutations (Fowler et al. 2020). Other studies evaluated allele frequencies of PGR polymorphisms

in breast and ovarian cancers and identified some intronic variants (Ghali et al. 2020, Kanabekova et al. 2022). Therefore, in this paper we adopted a comprehensive approach to find and characterize PGR mutations, for the first time in literature, in terms of their effect on protein stability and pathogenicity. Majority of the PGR mutations were identified as benign, while R319C, R623H and R869H were predicted to be pathogenic. Particularly, the disruptions of ionic interactions due to R623H and R869H mutations could result in the loss of the proposed protective role of PGR in PAAD.

Tumor infiltrating lymphocytes (TILs) are key components of immunotherapy, which has become one of the most promising novel therapy approaches in cancer treatment in the past decades. Lymphocyte infiltration to the solid tumor microenvironment hints towards responsiveness to immunotherapy and better clinical outcome, which attributes a prognostic and predictive significance to TILs. The desmoplastic stroma, which is a unique part of pancreatic adenocarcinoma histology, is known to interact with immune and inflammatory cells (Nielsen, Mortensen and Detlefsen, 2016). Therefore, we next evaluated tumor infiltrating lymphocytes in pancreatic cancer in relation to PGR expression and found a significant correlation between higher infiltration levels of CD8+ T cells, dendritic cells, macrophages and neutrophils and PGR expression. Previously it was reported that patients with CD4+ and CD8+ tumors and higher count of CD4+ and CD8+ T-cells, as well as dendritic cells, have improved prognosis in pancreatic cancer (Fukunaga et al., 2004; Ino et al., 2013). Macrophages are abundantly found in the pancreatic tumor stroma and different macrophage subsets (namely M1 and M2) are suggested to play paradoxical roles in pancreatic tumorigenesis, where M1 macrophages acts as pro-inflammatory immune cells, while M2 macrophages display anti-inflammatory and antitumor properties (Lankadasari et al., 2019; Yang et al., 2021).

5. Conclusion

In conclusion, the identification of the progesterone receptor (PGR) as a potential therapeutic target in pancreatic cancer is a significant advancement. These results could lead to the creation of focused therapy approaches that target PGR activity and open up new therapeutic options. By employing computational techniques, this study broadens our understanding of the complex biological mechanisms controlling the development of pancreatic adenocarcinoma. Furthermore, PGR's diverse roles, which have been proposed to provide protection against pancreatic cancer, highlight the potential for novel treatment approaches that specifically target PGR signaling networks. Nonetheless, further clinical studies are needed to validate its prognostic significance and improve patient outcomes.

Acknowledgements

The results published in this study are based upon data generated by the TCGA Research Network: https://www.cancer.gov/tcga.

References

Abe, M., Yamashita, J. and Ogawa, M. (2000) ‘Medroxyprogesterone acetate inhibits human pancreatic carcinoma cell growth by inducing apoptosis in association with Bcl-2

phosphorylation', Cancer, 88(9), pp. 2000–2009. Available at: https://doi.org/10.1002/(SICI)1097-0142(20000501)88:9<2000::AID-CNCR4>3.0.CO;2-B.

Alrushaid, N. et al. (2023) 'Progress and Perspectives in Colon Cancer Pathology, Diagnosis, and Treatments', Diseases, 11(4), p. 148. Available at: https://doi.org/10.3390/diseases11040148.

Benz, C., Hollander, C. and Miller, B. (1986) 'Endocrine-responsive Pancreatic Carcinoma: Steroid Binding and Cytotoxicity Studies in Human Tumor Cell Lines1', Cancer Research, 46(5), pp. 2276–2281.

Bertino, J.R. et al. (2011) 'Targeting tumors that lack methylthioadenosine phosphorylase (MTAP) activity', Cancer Biology & Therapy, 11(7), pp. 627–632. Available at: https://doi.org/10.4161/cbt.11.7.14948.

Boyuk, G., Yigit, A.A. and Aydogan, I. (2018) 'Co-culture of rat luteal cells with islet cells enhances islet viability and revascularization', In Vitro Cellular & Developmental Biology - Animal, 54, pp. 640–647. Available at: https://doi.org/10.1007/s11626-018-0286-y.

Carroll, J.S. et al. (2017) 'Deciphering the divergent roles of progestogens in breast cancer', Nature Reviews Cancer, 17(1), pp. 54–64. Available at: https://doi.org/10.1038/nrc.2016.116.

Cerami, E. et al. (2012) 'The cBio Cancer Genomics Portal: An Open Platform for Exploring Multidimensional Cancer Genomics Data', Cancer Discovery, 2(5), pp. 401–404. Available at: https://doi.org/10.1158/2159-8290.CD-12-0095.

Chandrashekar DS, Karthikeyan SK, Korla PK, et al. UALCAN: An update to the integrated cancer data analysis platform. *Neoplasia*. 2022;25:18-27. doi:10.1016/j.neo.2022.01.001

Cheng J, Randall A, Baldi P. (2006) Prediction of protein stability changes for single-site mutations using support vector machines. Proteins;62(4):1125-1132. doi:10.1002/prot.20810

Collins, M.A. et al. (2012) 'Metastatic Pancreatic Cancer Is Dependent on Oncogenic Kras in Mice', PLOS ONE, 7(12), p. e49707. Available at: https://doi.org/10.1371/journal.pone.0049707.

Doglioni, C. et al. (1990) 'Immunocytochemical localization of progesterone receptors in endocrine cells of the human pancreas.', The American Journal of Pathology, 137(5), pp. 999–1005.

Edwards, D.P. (2000) 'The Role of Coactivators and Corepressors in the Biology and Mechanism of Action of Steroid Hormone Receptors', Journal of Mammary Gland Biology and Neoplasia, 5(3), pp. 307–324. Available at: https://doi.org/10.1023/A:1009503029176.

Escriva, H., Bertrand, S. and Laudet, V. (2004) 'The evolution of the nuclear receptor superfamily', Essays in Biochemistry. Edited by I.J. McEwan, 40, pp. 11–26. Available at: https://doi.org/10.1042/bse0400011.

Fowler AM, Salem K, DeGrave M, et al. Progesterone Receptor Gene Variants in Metastatic Estrogen Receptor Positive Breast Cancer. *Horm Cancer*. 2020;11(2):63-75. doi:10.1007/s12672-020-00377-3

Fukunaga, A. et al. (2004) 'CD8+ Tumor-Infiltrating Lymphocytes Together with CD4+ Tumor-Infiltrating Lymphocytes and Dendritic Cells Improve the Prognosis of Patients with Pancreatic Adenocarcinoma', Pancreas, 28(1), p. e26.

Ghali RM, Al-Mutawa MA, Ebrahim BH, et al. Progesterone Receptor (PGR) Gene Variants Associated with Breast Cancer and Associated Features: a Case-Control Study. *Pathol Oncol Res*. 2020;26(1):141-147. doi:10.1007/s12253-017-0379-z

Goncharov, A.I. et al. (2017) 'Progesterone inhibits proliferation and modulates expression of proliferation—Related genes in classical progesterone receptor-negative human BxPC3 pancreatic adenocarcinoma cells', The Journal of Steroid Biochemistry and Molecular Biology, 165, pp. 293–304. Available at: https://doi.org/10.1016/j.jsbmb.2016.07.007.

Graham, J.D. and Clarke, C.L. (1997) 'Physiological Action of Progesterone in Target Tissues*', Endocrine Reviews, 18(4), pp. 502–519. Available at: https://doi.org/10.1210/edrv.18.4.0308.

Győrffy, B. (2024) 'Transcriptome-level discovery of survival-associated biomarkers and therapy targets in non-small-cell lung cancer', British Journal of Pharmacology, 181(3), pp. 362–374. Available at: https://doi.org/10.1111/bph.16257.

Hayashi, A., Hong, J. and Iacobuzio-Donahue, C.A. (2021) 'The pancreatic cancer genome revisited', Nature Reviews Gastroenterology & Hepatology, 18(7), pp. 469–481. Available at: https://doi.org/10.1038/s41575-021-00463-z.

Ino, Y. et al. (2013) 'Immune cell infiltration as an indicator of the immune microenvironment of pancreatic cancer', British Journal of Cancer, 108(4), pp. 914–923. Available at: https://doi.org/10.1038/bjc.2013.32.

Jaffee, E.M. et al. (2002) 'Focus on pancreas cancer', Cancer Cell, 2(1), pp. 25–28. Available at: https://doi.org/10.1016/S1535-6108(02)00093-4.

Jones, S. et al. (2008) 'Core Signaling Pathways in Human Pancreatic Cancers Revealed by Global Genomic Analyses', Science, 321(5897), pp. 1801–1806. Available at: https://doi.org/10.1126/science.1164368.

Kanabekova P, Al-Awadi AM, Bauyrzhanova Z, Tahtouh T, Sarray S, Almawi WY. Genetic variation in progesterone receptor gene and ovarian cancer risk: A case control study. *Gene*. 2022;820:146288. doi:10.1016/j.gene.2022.146288

Lankadasari, M.B. et al. (2019) 'TAMing pancreatic cancer: combat with a double edged sword', Molecular Cancer, 18(1), p. 48. Available at: https://doi.org/10.1186/s12943-019-0966-6.

Li, T. et al. (2020) 'TIMER2.0 for analysis of tumor-infiltrating immune cells', Nucleic Acids Research, 48(W1), pp. W509–W514. Available at: https://doi.org/10.1093/nar/gkaa407.

Liu X, Li C, Mou C, Dong Y, Tu Y. dbNSFP v4: a comprehensive database of transcript-specific functional predictions and annotations for human nonsynonymous and splice-site SNVs. Genome Med. 2020;12(1):103. Published 2020 Dec 2. doi:10.1186/s13073-020-00803-9

Maitra, A., Kern, S.E. and Hruban, R.H. (2006) 'Molecular pathogenesis of pancreatic cancer', Best Practice & Research Clinical Gastroenterology, 20(2), pp. 211–226. Available at: https://doi.org/10.1016/j.bpg.2005.10.002.

Mileshkin, L.R. et al. (2016) 'Phase II study of anastrozole in recurrent estrogen (ER) / progesterone (PR) positive endometrial cancer: The PARAGON trial—ANZGOG 0903.', Journal of Clinical Oncology, 34(15_suppl), pp. 5520–5520. Available at: https://doi.org/10.1200/JCO.2016.34.15_suppl.5520.

Mulac-Jericevic, B. and Conneely, O.M. (2004) 'Reproductive tissue selective actions of progesterone receptors', Reproduction, 128(2), pp. 139–146. Available at: https://doi.org/10.1530/rep.1.00189.

Nielsen, M.F.B., Mortensen, M.B. and Detlefsen, S. (2016) 'Key players in pancreatic cancer-stroma interaction: Cancer-associated fibroblasts, endothelial and inflammatory cells', World Journal of Gastroenterology, 22(9), pp. 2678–2700. Available at: https://doi.org/10.3748/wjg.v22.i9.2678.

Redston, M.S. et al. (1994) 'p53 Mutations in Pancreatic Carcinoma and Evidence of Common Involvement of Homocopolymer Tracts in DNA Microdeletions1', Cancer Research, 54(11), pp. 3025–3033.

Scarpin, K.M. et al. (2009) 'Progesterone Action in Human Tissues: Regulation by Progesterone Receptor (PR) Isoform Expression, Nuclear Positioning and Coregulator Expression', Nuclear Receptor Signaling, 7(1), p. nrs.07009. Available at: https://doi.org/10.1621/nrs.07009.

Selvan, R.S., Metzgar, R.S. and Petrow, V. (1992) 'Growth modulatory effects of some 6-methylenic steroids on human and hamster pancreatic adenocarcinoma cells in vitro', Drug design and discovery, 9(2), pp. 119–133.

Shi, T. and Gao, G. (2022) 'Identify potential prognostic indicators and tumor-infiltrating immune cells in pancreatic adenocarcinoma', Bioscience Reports, 42(2), p. BSR20212523. Available at: https://doi.org/10.1042/BSR20212523.

Siegel, P.M. and Massagué, J. (2003) 'Cytostatic and apoptotic actions of TGF-β in homeostasis and cancer', Nature Reviews Cancer, 3(11), pp. 807–820. Available at: https://doi.org/10.1038/nrc1208.

Targarona, E.M. et al. (1991) 'Is exocrine pancreatic cancer a hormone-dependent tumor? A study of the existence of sex hormone receptors in normal and neoplastic pancreas', Hepato-gastroenterology, 38(2), pp. 165–169.

Teunissen, S.C.C.M. et al. (2007) 'Symptom Prevalence in Patients with Incurable Cancer: A Systematic Review', Journal of Pain and Symptom Management, 34(1), pp. 94–104. Available at: https://doi.org/10.1016/j.jpainsymman.2006.10.015.

Thomas, A. et al. (2010) 'Adjuvant Therapy in Pancreatic Cancer', Digestive Diseases, 28(4–5), pp. 684–692. Available at: https://doi.org/10.1159/000320099.

Uhlén, M., Fagerberg, L., Hallström, B. M., Lindskog, C., Oksvold, P. et al. (2015). Proteomics. Tissue-based map of the human proteome. *Science, 347(6220),* 1260419. DOI 10.1126/science.1260419.

van Kruchten, M. et al. (2015) 'Hormone receptors as a marker of poor survival in epithelial ovarian cancer', Gynecologic Oncology, 138(3), pp. 634–639. Available at: https://doi.org/10.1016/j.ygyno.2015.06.032.

Varadi M, Bertoni D, Magana P, et al. AlphaFold Protein Structure Database in 2024: providing structure coverage for over 214 million protein sequences. *Nucleic Acids Res*. 2024;52(D1):D368-D375. doi:10.1093/nar/gkad1011

Viale, G. et al. (1992) 'Progesterone receptor immunoreactivity in pancreatic endocrine tumors. An immunocytochemical study of 156 neuroendocrine tumors of the pancreas, gastrointestinal and respiratory tracts, and skin', Cancer, 70(9), pp. 2268–2277. Available at: https://doi.org/10.1002/1097-0142(19921101)70:9<2268::AID-CNCR2820700910>3.0.CO;2-X.

Yang, Y. et al. (2021) 'M2 Macrophage-Derived Exosomes Promote Angiogenesis and Growth of Pancreatic Ductal Adenocarcinoma by Targeting E2F2', Molecular Therapy, 29(3), pp. 1226–1238. Available at: https://doi.org/10.1016/j.ymthe.2020.11.024.

Yao, Y. and Dai, W. (2014) 'Genomic Instability and Cancer', Journal of Carcinogenesis & Mutagenesis, 5, p. 1000165. Available at: https://doi.org/10.4172/2157-2518.1000165.

Tables and Figures

Table 1: Top 20 genes with the highest expression in PGR-low group

Gene	*Cytoband*	*NCBI Gene Summary*	*p-Value*
ACTB	7p22.1	Actin B, cytoskeletal protein	3.15e-3
ACTG1	17q25.3	Actin G1, cytoskeletal protein	2.21e-4
GAPDH	12p13.31	Glyceraldehyde-3-phosphate dehydrogenase	2.36e-8
TMSB4XP8	4q22.1	Pseudogene	0.0172
UBC	12q24.3	Ubiquitin C, polyubiquitin precursor	8.83e-3
HLA-B	6p21.33	Antigen-presenting major histocompatibility complex class I component	4.15e-3
HLA-A	6p22.1	Antigen-presenting major histocompatibility complex class I component	2.36e-4
RPL8	8q24.3	Ribosomal protein	8.31e-6
PKM	15q23	Pyruvate kinase, involved in glycolysis	8.69e-5
CTSD	11p15.5	Cathepsin D, member of the A1 family of peptidases	2.91e-4
S100A6	1q21.3	Calcium binding protein	6.63e-7
KRT8	12q13.13	Keratin 8	2.39e-6
ATP1A1	1p13.1	Sodium/potassium-transporting ATPase subunit	3.04e-3
RPS11	19q13.3	Small ribosomal subunit protein	0.0169
RPLP0	12q24.23	Large ribosomal subunit protein	1.49e-3
RPS3	11q13.4	Small ribosomal subunit protein	5.49e-3
RACK1	5q35.3	Receptor for activated c kinase, ribosomal protein	3.46e-3

PABPC1	8q22.3	Poly(A) binding protein	0.0241
HLA-C	6p21.33	Antigen-presenting major histocompatibility complex class I component	1.92e-3
RPL19	17q12	Ribosomal protein	1.81e-3

Table 2: Top 20 genes with the highest expression in PGR-high group

Gene	*Cytoband*	*NCBI Gene Summary*	*p-Value*
SPARC	5q33.1	Cysteine-rich acidic matrix-associated protein	1.77e-3
PSAP	10q22.1	Prosaposin, facilitates the catabolism of glycosphingolipids	4.30e-7
GNAS	20q13.32	Guanine nucleotide binding protein	2.34e-3
IGFBP5	2q35	Insulin-like growth factor-binding protein	1.04e-4
VIM	10p13	Vimentin, type iii intermediate filament protein	2.41e-3
LUM	12q21.33	Lumican, member of the small leucine-rich proteoglycan (slrp) family	0.0133
APP	21q21.3	Amyloid beta precursor protein	1.30e-7
ITGB1	10p11.22	Integrin subunit beta, membrane receptor involved in cell adhesion and recognition	0.0185
IGFBP7	4q12	Insulin-like growth factor-binding protein	1,56e-6
A2M	12p13.31	Alpha-2-macroglobulin, a protease inhibitor and cytokine transporter	1.47e-7
DCN	12q21.33	Decorin, member of the small leucine-rich proteoglycan family of proteins	5.12e-4
HSPA8	11q24.1	Member of the heat shock protein 70 family	1.68e-4
CLU	8p21.1	Clusterin, chaperone involved in cell death, tumor progression neurodegenerative disorders	7.06e-5
IGFBP4	17q21.2	Insulin-like growth factor-binding protein	0.0230

COL4A2	13q34	Collagen type IV, major structural component of basement membranes	8.06e-3
EIF4G2	11p15.4	Eukaryotic translation initiation factor	2.10e-6
COL4A1	13q34	Collagen type IV, major structural component of basement membranes	3.01e-4
TIMP2	17q25.3	Metallopeptidase inhibitor, involved in degradation of the extracellular matrix	8.40e-7
TIMP3	22q12.3	Metallopeptidase inhibitor, involved in degradation of the extracellular matrix	3.55e-5
THBS1	15q14	Thrombospondin, an adhesive glycoprotein mediating cell-to-cell/matrix interactions	3.99e-5

Table 3: List of PGR mutations in PAAD

	Protein stability		Pathogenicity		Localization
Mutation	**Prediction**	**ΔΔG**	**Prediction**	**MetaRNN Score**	Progesterone receptor domain
p.A218V	Decrease	-0.558738	Benign	0.061644	Progesterone receptor domain
p.D225G	Decrease	-1.431063	Benign	0.083149	Progesterone receptor domain
p.R242Q	Decrease	-0.132059	Benign	0.1240228	Progesterone receptor domain
p.A247T	Decrease	-0.955131	Benign	0.023921	Progesterone receptor domain
p.R319C	Decrease	-0.871485	Pathogenic	0.933363	Progesterone receptor domain
p.S344T	Decrease	-0.493508	Benign	0.00192	Progesterone receptor domain
p.Y374S	Decrease	-0.534782	Benign	0.121555	Progesterone receptor domain
p.Q556H	Decrease	-1.054914	Benign	0.33521017	Progesterone receptor domain
p.R623H	Decrease	-0.745942	Pathogenic	0.9873885	Zinc-finger domain

p.R788Q	Decrease	-0.534782	Benign	0.225403	Hormone receptor domain
p.R869H	Decrease	-1.054914	Pathogenic	0.902717	Hormone receptor domain

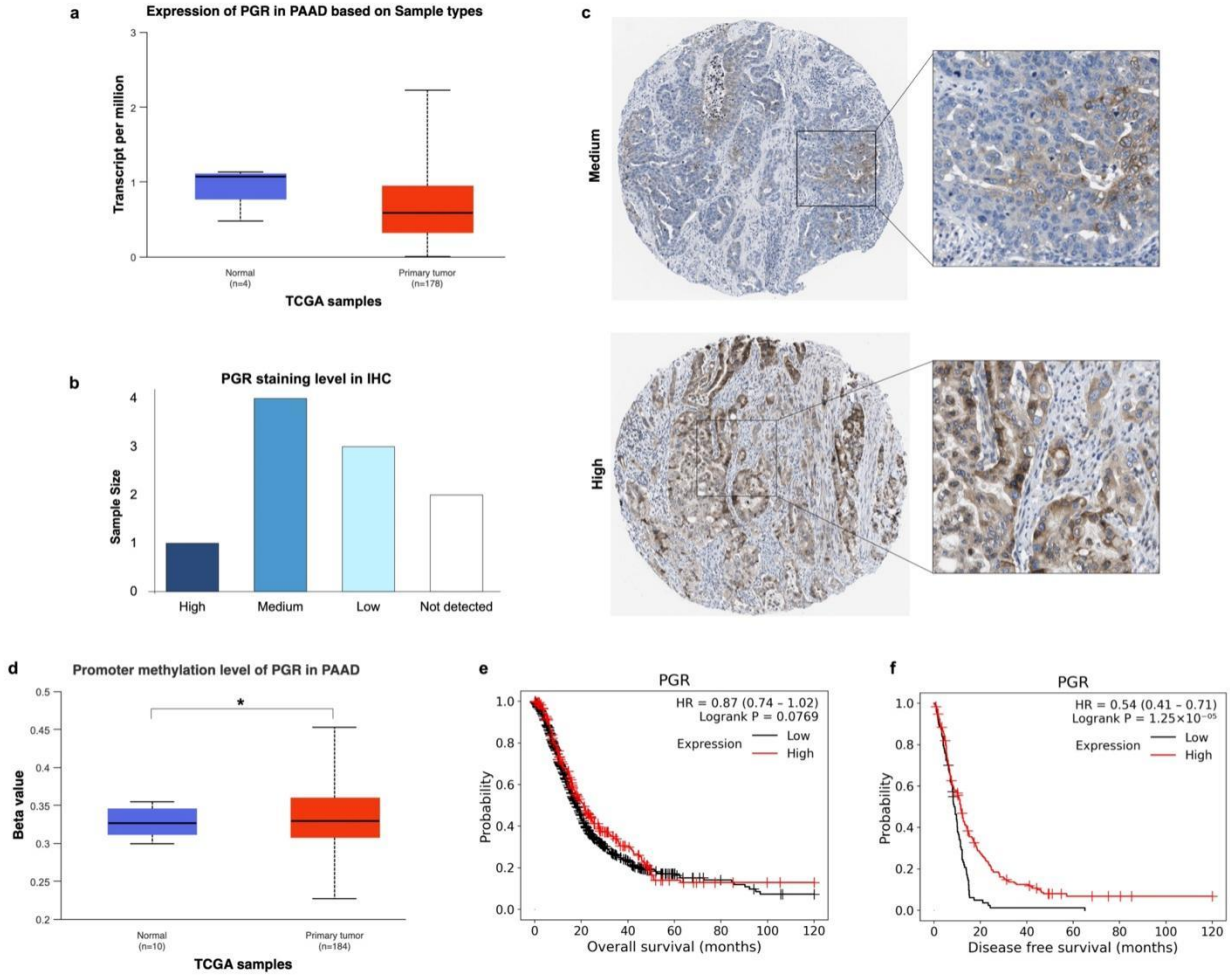

Figure 1. Comparison of PGR expression between tumor and normal tissues (a), quantification of PGR protein expression levels based on immunohistochemistry staining intensities (b) and immunohistochemistry images of PAAD tumor samples (c). Promoter methylation level of PGR in PAAD tumor and adjacent normal tissues (d). Overall (e) and disease free (f) survival probability of PAAD patients in relation to PGR mRNA expression levels. (*p<0.05)

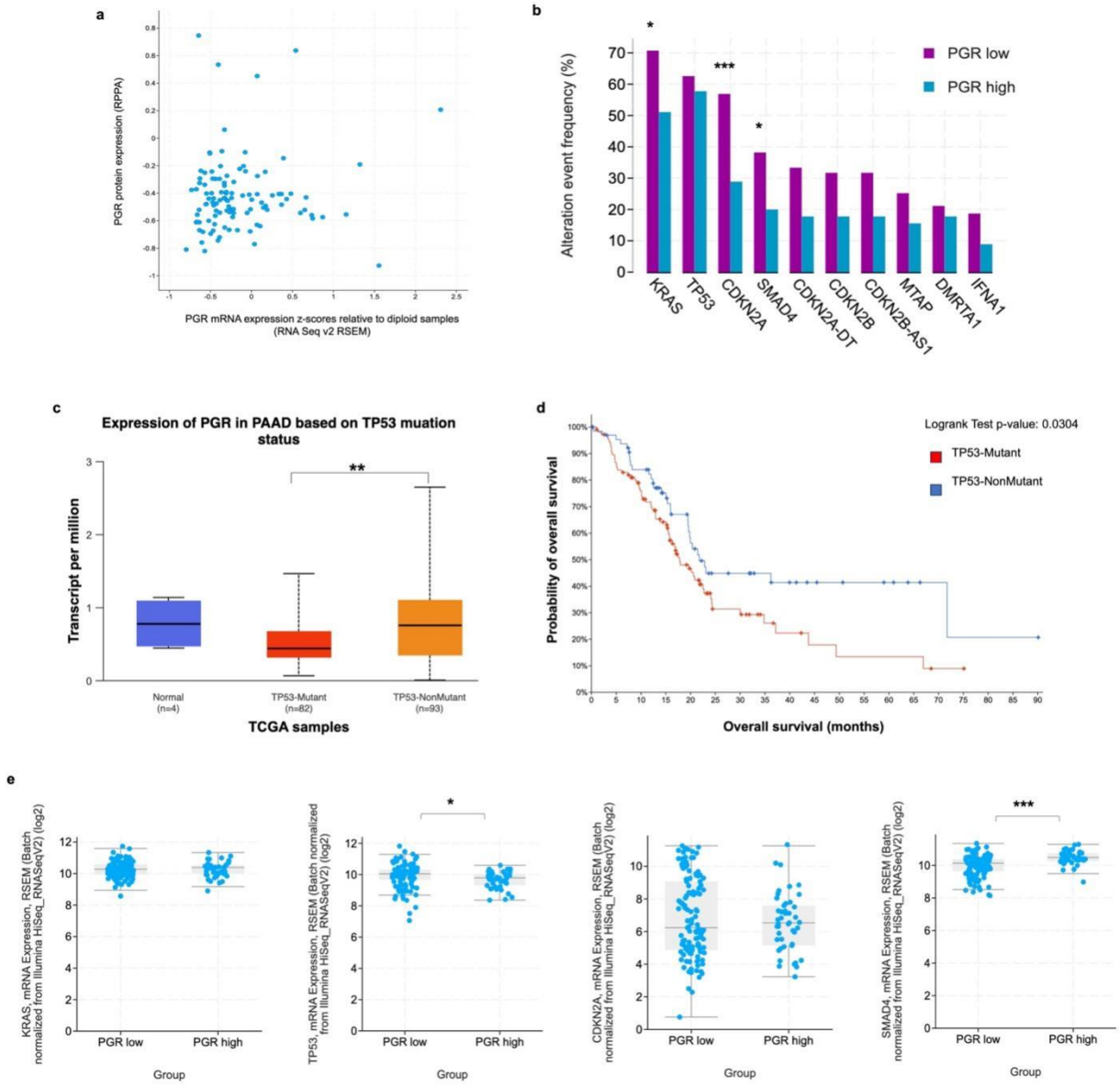

Figure 2. mRNA expression levels of PGR within PAAD patient cohort in relation to PGR protein expression (a). Top 10 genes with highest frequency of genomic alterations in patients with low or high PGR expression (b). Expression level of PGR in TP53-mutant and -nonmutant tissue samples (c). Kaplan-Meier survival analysis of patients with mutant/nonmutant TP53 (d). mRNA expression levels of the most frequently altered genes in relation to PGR expression in PAAD (e). (*p<0.05, **p<0.01, ***p<0.001)

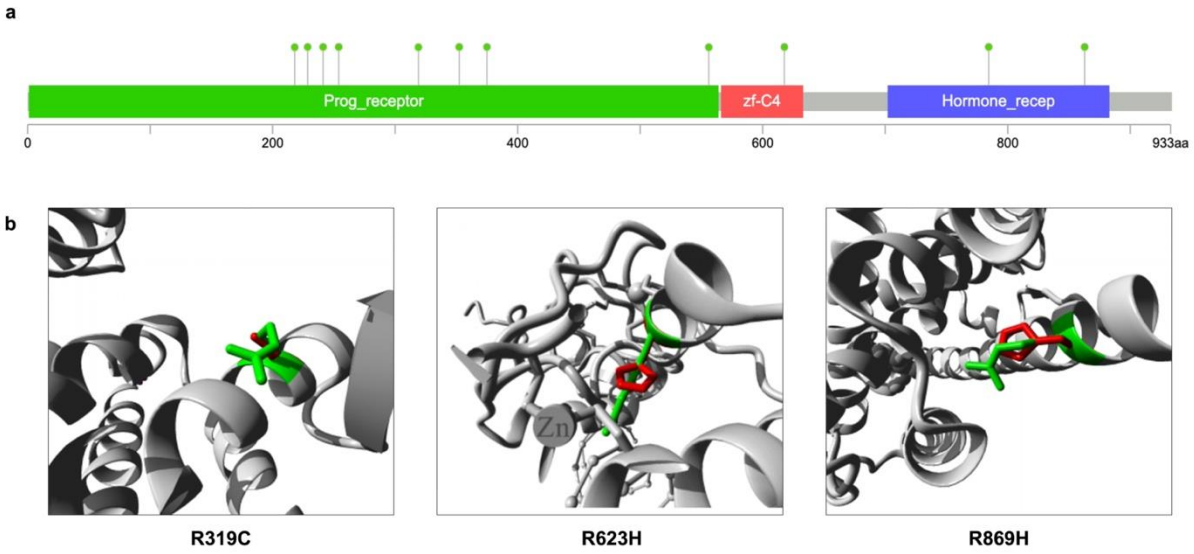

Figure 3. Missense amino acid substitutions in PGR gene in pancreatic adenocarcinoma (PAAD) (a). Progesterone receptor domain (green), zinc-finger domain (pink) and hormone receptor domain (blue) are indicated. Close-up views of the potentially pathogenic PGR mutations (b). The protein is shown in grey, while the side chains of the wild-type and the mutant residue are in green and red respectively. Zinc ion is indicated as Zn.

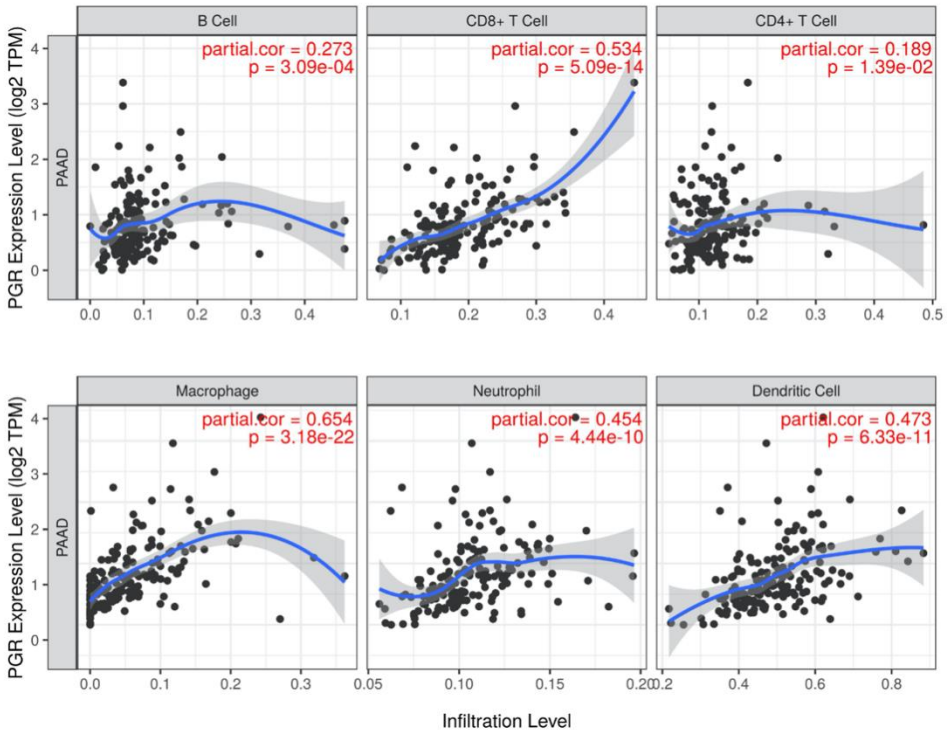

Figure 4: Tumor infiltration levels in relation to PGR expression in PAAD. Higher expression of PGR correlates significantly with increased infiltration levels in CD8+ T-cells, macrophages, neutrophils and dendritic cell subsets. Each dot represents a patient.