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Hormone Receptor Expression Varies by Molecular Subtype, Age, and Sex in Pheochromocytoma and Paraganglioma: A Transcriptomic Analysis

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Abstract

Purpose The role of hormone receptor (HR) signaling in pheochromocytoma and paraganglioma (PCPG) is incompletely defined. We characterized HR expression across PCPG molecular subtypes and its clinicopathologic, transcriptional, and outcome associations.

Methods RNA-sequencing data from 151 primary tumors (one per patient) in The Cancer Genome Atlas were analyzed for ESR1, ESR2, GPER1, PGR, AR, LHCGR, and the glucocorticoid receptor gene NR3C1. Expression was compared by molecular cluster (pseudohypoxia, kinase signaling, Wnt-altered), age, sex, and metastatic status using non-parametric tests. Event-free survival (EFS) was assessed with Cox models, and ESR1- and AR-associated programs with gene set enrichment analysis. Cluster patterns were tested in the independent E-MTAB-733 cohort.

Results Pseudohypoxia tumors had higher ESR1 and PGR and lower LHCGR expression than other clusters (all $p < 0.001$), a pattern seen in both sexes. Kinase-signaling tumors had lower GPER1 and AR expression. NR3C1 did not differ by cluster. ESR1 and PGR decreased and LHCGR increased with age, predominantly in male patients. Lower LHCGR in pseudohypoxia tumors and lower AR in kinase-signaling tumors were associated with metastatic disease. No receptor was associated with EFS; overall survival could not be modeled (five deaths). In pseudohypoxia tumors, ESR1-high cases showed immune-enriched programs and AR-high cases developmental programs with oxidative phosphorylation depletion. Externally, cluster differences in PGR, LHCGR, AR, and GPER1, but not ESR1, were reproduced.

Conclusion HR expression in PCPG varies by molecular subtype, age, and sex, and selected receptors track with metastatic behavior and distinct transcriptional programs. Protein-level validation and larger outcome-annotated cohorts are needed.

Keywords Pheochromocytoma; Paraganglioma; Estrogen receptor; Androgen receptor; Luteinizing hormone receptor; Molecular subtypes

Introduction

Pheochromocytomas and paragangliomas (PCPGs) are rare neuroendocrine tumors with an incidence of 2 to 8 per million that often occur in the third through fifth decades of life [1, 2]. Most PCPGs do not metastasize, though the sequelae of catecholamine and metanephrine hypersecretion contribute to morbidity and mortality [1]. With limited treatment options, metastatic PCPGs, defined by spread to non-chromaffin tissues, have a poor prognosis, with fewer than 50% of patients surviving five years [3]. Up to 40% of PCPGs harbor germline mutations in known susceptibility genes, making them the most heritable solid tumor [1, 4]. More than 20 germline and somatic driver genes have been described and can be grouped into three molecular clusters based on their downstream signaling effects: pseudohypoxia (cluster 1), kinase signaling (cluster 2), and Wnt-altered (cluster 3) [5, 6]. Each cluster shows characteristic clinicopathologic features and outcomes, which are increasingly used to guide individualized surveillance and therapy.

In other endocrine-driven cancers, circulating estrogen, progesterone, and testosterone influence tumorigenesis and progression, and selective hormone modulation is an established therapeutic strategy [7–9]. Wang et al. evaluated the effects of sex hormones on PCPGs using primary tumors from 38 patients and demonstrated dose-dependent in vitro anti-tumor effects of estrogen, testosterone, and GPER agonism [10]. Adrenal chromaffin and pheochromocytoma cells express high-affinity testosterone binding sites, activation of which increases cytosolic calcium, decreases catecholamine secretion, and induces apoptosis, further supporting a functional role for hormone signaling in chromaffin-derived cells [11]. Key hormone receptor (HR) genes include ESR1 and ESR2, which encode the canonical nuclear estrogen receptors ERα and ERβ; GPER1, which encodes the G protein-coupled estrogen receptor that mediates

rapid non-genomic estrogen signaling; PGR, encoding the progesterone receptor; AR, encoding the androgen receptor; and LHCGR, encoding the luteinizing hormone/chorionic gonadotropin receptor that regulates gonadal and adrenal steroidogenesis [12, 13].

Clinical observations also support a role for the sex hormonal milieu in PCPG behavior. Pheochromocytomas are more frequently diagnosed in females, who report more signs and symptoms than males regardless of biochemical phenotype or tumor presentation [14, 15]. These differences may relate in part to sex-specific catecholamine receptor sensitivity, with females having greater α2- and β2-adrenergic receptor responsiveness than males [15, 16]. In contrast, a meta-analysis of metastatic PCPGs demonstrated higher mortality in male patients and in those with synchronous metastases, suggesting that sex-linked factors may contribute to outcome disparities in advanced disease [17]. Despite the central role of HR pathways in other endocrine malignancies, their expression and function in PCPG remain poorly characterized. We therefore investigated HR expression patterns and their clinicopathologic and prognostic relevance across PCPG molecular clusters using The Cancer Genome Atlas (TCGA) and E-MTAB-733 datasets.

Materials and methods

Data acquisition and preprocessing

Bulk RNA-sequencing data (STAR-counts workflow) for PCPG were obtained from TCGA via the Genomic Data Commons (GDC) using the TCGAbiolinks R package. Analyses were restricted to primary tumor samples (TCGA sample type 01), one per patient; additional tumor (sample types 05 and 06) and normal tissue (sample type 11) aliquots were excluded. Raw counts of all primary tumors were normalized to log2 counts per million (CPM) using edgeR with trimmed mean of M-values (TMM) normalization and a prior count of 1 for variance stabilization.

Six sex hormone–related receptor genes were selected based on their established roles in hormone signaling: ESR1 (estrogen receptor α), ESR2 (estrogen receptor β), GPER1 (G protein-coupled estrogen receptor), PGR (progesterone receptor), AR (androgen receptor), and LHCGR (luteinizing hormone/chorionic gonadotropin receptor), the latter included given prior work implicating LHCGR in PCPG biology [18]. The glucocorticoid receptor gene NR3C1 was analyzed separately because adrenal glucocorticoids regulate epinephrine synthesis in chromaffin cells [19]. Gene identifiers were standardized by removing Ensembl version numbers and mapping to HGNC gene symbols.

Clinical annotations, including molecular cluster assignments, sex, age at diagnosis, tumor type, metastatic status, and clinically aggressive status, were obtained from TCGA clinical data and the curated dataset published by Fishbein et al. [6]. These annotations were matched to expression data at the patient level using the first 12 characters of the TCGA barcode, so that each patient contributed a single primary tumor to all analyses.

Statistical analysis of gene expression

Because expression values were not normally distributed, non-parametric methods were used throughout. Expression was compared across the three molecular clusters using the Kruskal–Wallis test with Dunn's post hoc tests (rstatix package); omnibus Kruskal–Wallis p-values are reported in the text. Associations with sex were evaluated using the Wilcoxon rank-sum test, and correlations between expression and age using Spearman rank correlation, both overall and stratified by sex. To evaluate whether cluster-differential patterns were consistent across sexes, expression was additionally compared across clusters within each sex using pairwise Wilcoxon rank-sum tests. As a comparator for age-related changes in a hormonally driven tumor, the same receptors were correlated with age in TCGA prostate adenocarcinoma (PRAD) primary tumors

(one per patient) processed identically. Associations with metastatic or clinically aggressive disease were evaluated using Wilcoxon rank-sum tests within molecular clusters. Post hoc and pairwise comparisons were adjusted using the Benjamini–Hochberg method, with adjusted $p \leq 0.05$ considered significant. Supplementary Figs. S1–S7 are provided in Online Resource 1.

For external validation, normalized robust multi-array average (RMA) expression data (Affymetrix HG-U133 Plus 2.0) and published transcriptome class annotations for the E-MTAB-733 cohort [20] were obtained from ArrayExpress. Annotations were harmonized into broader biologic classes rather than re-derived by unsupervised clustering: C1A and C1B as pseudohypoxia-related, C2A as kinase-signaling-related, and C2B as Wnt-altered-like. C2C samples were excluded because later PCPG subtype frameworks suggest that C2C aligns more closely with admixture or confounded biology than with canonical Wnt-altered disease [21–23]. Expression was compared across harmonized classes using the Kruskal–Wallis test.

Differential expression and gene set enrichment analysis

Within the pseudohypoxia cluster (cluster 1), tumors were dichotomized at the median log2 CPM of ESR1 and, separately, of AR. Differential expression (ESR1-high vs. ESR1-low; AR-high vs. AR-low) was computed using the limma-voom framework, which models the mean–variance relationship of RNA-sequencing data and applies empirical Bayes variance moderation [24, 25]. Genes were ranked by log2 fold change and annotated with HGNC symbols; when multiple Ensembl identifiers mapped to the same symbol, the one with the highest absolute fold change was retained. Pre-ranked gene set enrichment analysis (GSEA) of Gene Ontology Biological Process pathways was performed with gseGO in clusterProfiler [26] (fixed random seed), which implements the fast gene set enrichment algorithm [27, 28]. Given the redundancy of GO annotations, pathways were filtered at an adjusted $p < 0.01$, ranked by absolute

normalized enrichment score (NES), and restricted to top representative pathways. To compare ESR1- and AR-associated programs, results were aligned by pathway and the difference in NES was calculated ($\Delta NES = NES_{ESR1} - NES_{AR}$).

Survival analysis

Vital status and overall survival (OS) time were obtained from TCGA clinical data. Only five deaths occurred (two in cluster 1), which precluded meaningful OS modeling; OS is therefore reported descriptively. Because event-free survival (EFS) was not consistently available in TCGA, EFS endpoints were derived from the curated Fishbein et al. dataset [6]: clinically aggressive and/or metastatic disease (16 patients, 15 with evaluable event-free time) and distant metastasis (11 patients, 10 with evaluable event-free time). Events were coded as 1 and censored observations as 0. Univariate Cox proportional hazards models (Efron method for ties) were fitted at the patient level (one primary tumor per patient) to estimate hazard ratios (HRs) for each endpoint, overall and within each molecular cluster, with receptor expression modeled as a continuous variable; hazard ratios are reported per 1-unit increase in log2 CPM (i.e., per two-fold increase in expression). Proportional hazards assumptions were assessed using Schoenfeld residuals. Analyses used the survival package [29]. Because no open independent PCPG cohort with matched bulk expression and sufficiently complete survival data was available, time-to-event analyses were restricted to TCGA-PCPG, and external data were used only to replicate expression findings.

Data visualization and computational environment

Figures were generated with ggplot2, ggpubr, and pheatmap, and data were processed with dplyr and tidyr [30–34]. Fig. 2a was partially created using BioRender [35]. All analyses were performed in R (version 4.5.1). Code development was assisted by large language models,

including ChatGPT (OpenAI), Perplexity (Perplexity AI), and Claude (Anthropic); all outputs were reviewed and validated by the authors to ensure accuracy and reproducibility.

Results

Patient characteristics

A total of 178 tumor samples from 173 patients were available in the curated TCGA-PCPG dataset. After exclusion of 22 samples classified as cortical admixture and 5 additional samples without a cluster assignment (all from patients whose primary tumor was retained), 151 primary tumors from 151 patients were analyzed: 61 in the pseudohypoxia cluster, 68 in the kinase-signaling cluster, and 22 in the Wnt-altered cluster. Mean age at diagnosis was 43.4 years (SD 15.6) in cluster 1, 49.2 years (SD 15.1) in cluster 2, and 51.5 years (SD 12.5) in cluster 3. Male patients accounted for 44.3% (27/61) of cluster 1, 41.2% (28/68) of cluster 2, and 54.5% (12/22) of cluster 3 (67/151, 44.4% overall). Mean follow-up was 37.9 months (SD 49.9), 24.0 months (SD 24.1), and 33.2 months (SD 36.9), respectively. Five patients died during follow-up: two in cluster 1, one in cluster 2, and two in cluster 3 (Table 1).

Table 1 Patient and tumor characteristics by molecular cluster (patient level; one primary tumor per patient)

	Cluster 1 Pseudohypoxia	Cluster 2 Kinase signaling	Cluster 3 Wnt-altered	Total
Patients, n	61	68	22	151
Age at diagnosis, years				
Mean (SD)	43.4 (15.6)	49.2 (15.1)	51.5 (12.5)	47.2 (15.2)
Median	42	46.5	48.5	46
Range	19–75	19–83	33–74	19–83
Sex, n (%)				
Male	27 (44.3)	28 (41.2)	12 (54.5)	67 (44.4)
Female	34 (55.7)	40 (58.8)	10 (45.5)	84 (55.6)
Follow-up, months				
Mean (SD)	37.9 (49.9)	24.0 (24.1)	33.2 (36.9)	31.0 (38.6)
Median	23.8	16.9	23.2	19.6
Range	0.2–316.5	0.1–104.5	0.8–127.5	0.1–316.5
Disease status, n				
Metastatic disease	6	2	3	11
Clinically aggressive and/or metastatic	8	2	6	16
Deaths during follow-up				
Patients who died, n	2	1	2	5

Values are per patient (n = 151; one primary tumor each). Metastatic and clinically aggressive status are from the curated dataset of Fishbein et al.; metastatic status was unknown for 4 patients. SD, standard deviation

Hormone receptor expression by age and sex

Age at diagnosis was lower in cluster 1 than in clusters 2 and 3 (Kruskal–Wallis $p = 0.037$; Fig. S1). Across all clusters combined, ESR1 and PGR expression were inversely correlated with age ($\rho = -0.249$, $p = 0.002$; $\rho = -0.242$, $p = 0.003$), whereas LHCGR expression was positively correlated with age ($\rho = 0.172$, $p = 0.035$). No significant associations were observed for AR, ESR2, or GPER1 ($\rho = 0.003$, $p = 0.967$; $\rho = 0.120$, $p = 0.144$; $\rho = -0.043$, $p = 0.602$) (Fig. 1a).

When stratified by sex, no receptor was significantly associated with age in female patients. In male patients, ESR2 and LHCGR expression were positively correlated with age ($\rho = 0.301$, $p = 0.013$; $\rho = 0.254$, $p = 0.038$), whereas ESR1 and PGR expression were negatively correlated

183 with age ($\rho = -0.364$, $p = 0.002$; $\rho = -0.317$, $p = 0.009$). AR and GPER1 were not significantly
184 associated with age in either sex (all $p > 0.05$) (Fig. 1b). When cluster comparisons were
185 stratified by sex, cluster-differential expression of ESR1, PGR, and LHCGR was observed in
186 both female and male patients, whereas AR and GPER1 differed across clusters only in male
187 patients, with no significant adjusted pairwise difference in female patients (Fig. S2).

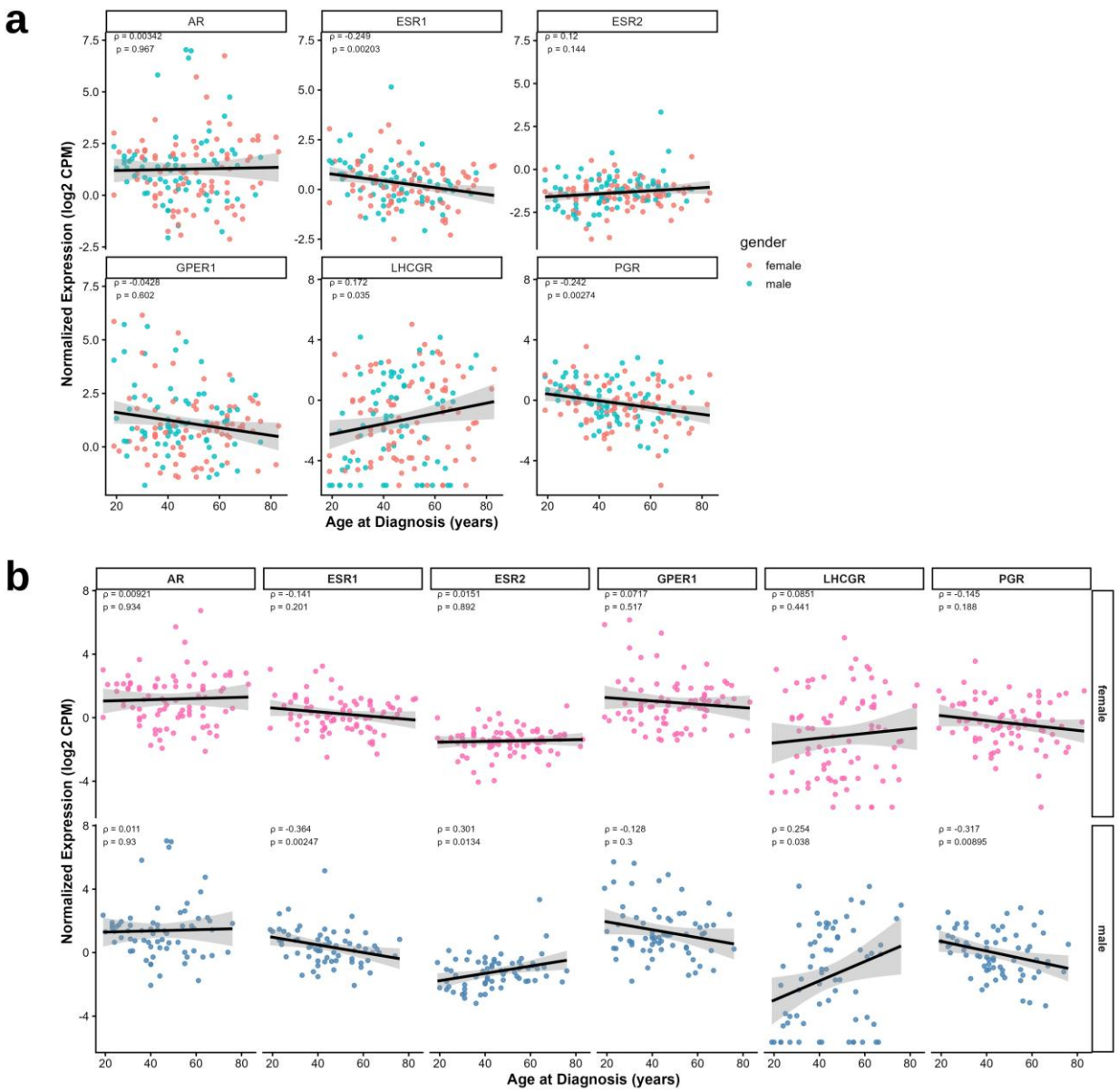


Fig. 1 Hormone receptor (HR) gene expression in PCPG tumors differs by age at diagnosis and sex. (a) Expression of AR, ESR1, ESR2, GPER1, LHCGR, and PGR versus age at diagnosis for all patients (n = 151; one primary tumor each), colored by sex. ESR1 and PGR expression were inversely correlated with age ($\rho = -0.249$, $p = 0.002$; $\rho = -0.242$, $p = 0.003$), whereas LHCGR expression was positively correlated with age ($\rho = 0.172$, $p = 0.035$). (b) HR expression versus age stratified by sex: female (top row) and male (bottom row). In male patients, ESR2 and

LHCGR expression were positively correlated with age, whereas ESR1 and PGR expression were negatively correlated with age. AR and GPER1 were not significantly associated with age in either sex. Abbreviations: AR, androgen receptor; ESR1/2, estrogen receptor 1/2; GPER1, G protein-coupled estrogen receptor 1; HR, hormone receptor; LHCGR, luteinizing hormone/choriogonadotropin receptor; PCPG, pheochromocytoma and paraganglioma; PGR, progesterone receptor

To determine whether these age associations reflected generalized age-related changes rather than tumor biology, the same six receptors were examined in TCGA-PRAD, chosen because the age effects in PCPG were concentrated in male patients and PRAD is a hormonally driven cancer [36, 37]. In PRAD, ESR1 expression was positively correlated with age ($\rho = 0.116$, $p = 0.013$) and GPER1 negatively correlated ($\rho = -0.120$, $p = 0.010$); AR, ESR2, LHCGR, and PGR were not significantly correlated with age (Fig. S3). These patterns differed from those in PCPG, suggesting that the PCPG associations reflect tumor-specific biology rather than generalized age-related changes.

Hormone receptor expression by molecular cluster

Cluster 1 tumors had higher expression of ESR1 and PGR (Kruskal–Wallis $p = 4.3 \times 10^{-7}$ and $p = 3.5 \times 10^{-7}$) and lower expression of LHCGR ($p = 2.6 \times 10^{-14}$) than clusters 2 and 3 (all Dunn-adjusted $p < 0.001$). GPER1 expression was lower in cluster 2 than in clusters 1 and 3 ($p = 1.9 \times 10^{-4}$), and AR expression was lower in cluster 2 than in cluster 1 ($p = 0.019$); ESR2 showed a non-significant trend toward higher expression in cluster 2 ($p = 0.067$) (Fig. 2a, b). NR3C1 expression did not differ across clusters (Kruskal–Wallis $\chi^2 = 4.96$, $df = 2$, $p = 0.084$) (Fig. S4) and was not associated with EFS overall or in any evaluable cluster (Online Resource 2, Table S1).

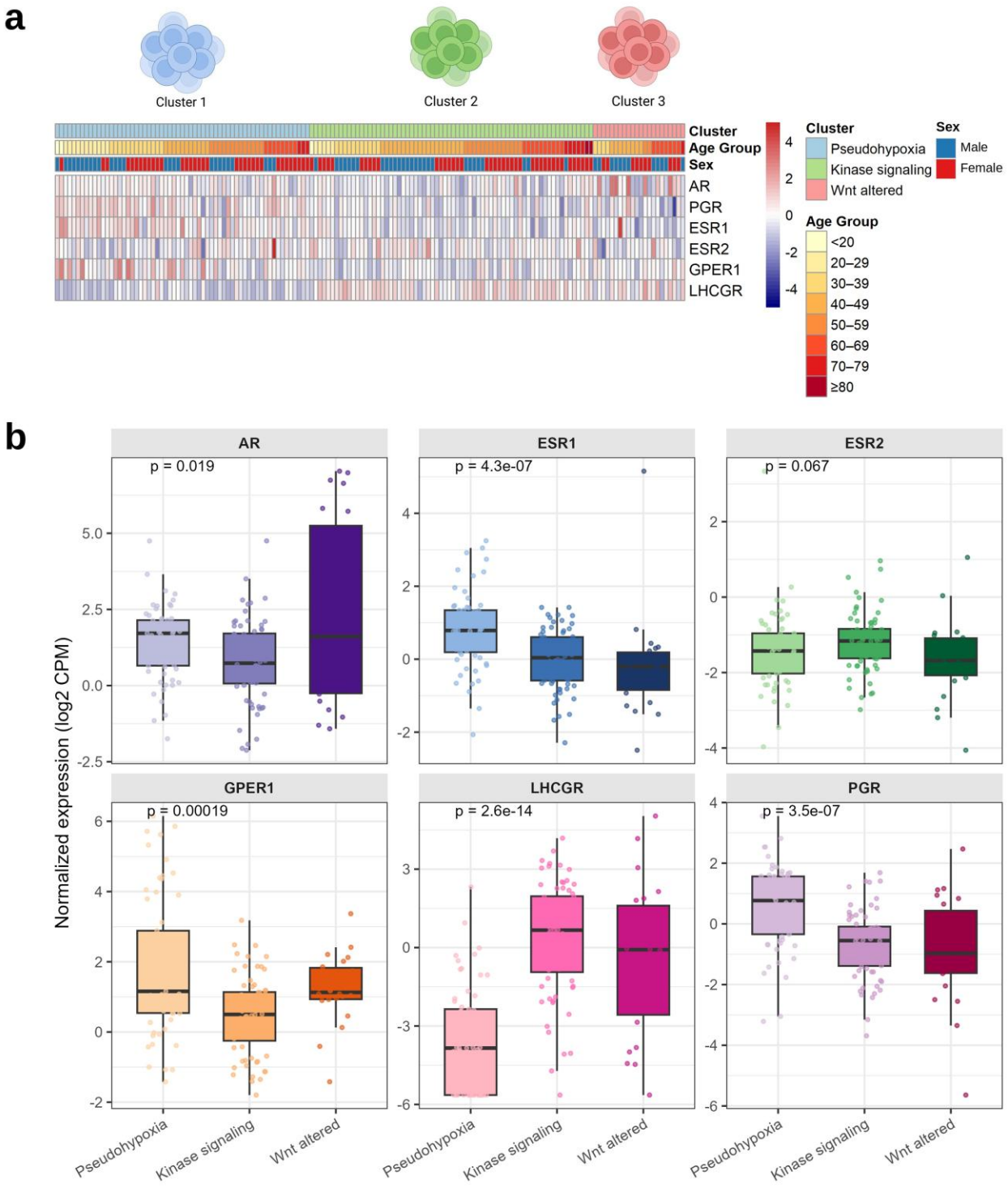
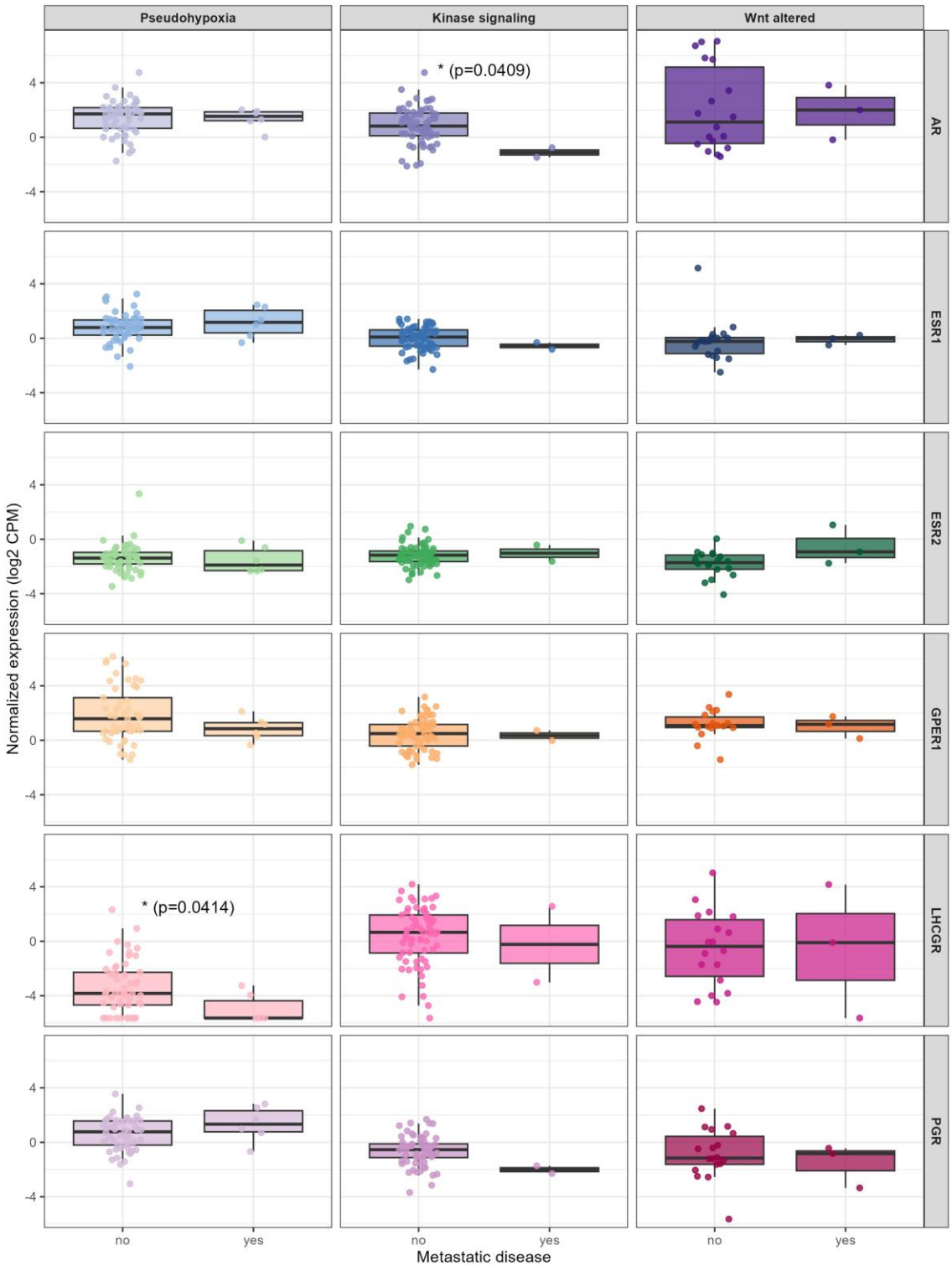


Fig. 2 Hormone receptor (HR) expression by molecular cluster in PCPG. (a) Heatmap of row-scaled HR expression by molecular cluster, age group, and sex. Partially created with BioRender.com. (b) HR expression by molecular cluster. ESR1 and PGR expression were higher

in cluster 1 than in clusters 2 and 3 (Kruskal–Wallis $p = 4.3 \times 10^{-7}$ and $p = 3.5 \times 10^{-7}$), and LHCGR expression was lower ($p = 2.6 \times 10^{-14}$). GPER1 expression was lower in cluster 2 than in clusters 1 and 3 ($p = 1.9 \times 10^{-4}$), and AR expression was lower in cluster 2 than in cluster 1 ($p = 0.019$). Pairwise comparisons used Dunn's test with Benjamini–Hochberg correction.

Abbreviations: AR, androgen receptor; ESR1/2, estrogen receptors 1 and 2; GPER1, G protein-coupled estrogen receptor 1; LHCGR, luteinizing hormone/choriogonadotropin receptor; PCPG, pheochromocytoma and paraganglioma; PGR, progesterone receptor

Within cluster 1, low LHCGR expression was associated with metastatic disease ($p = 0.041$). Within cluster 2, low AR expression was associated with metastatic disease ($p = 0.041$), although only two patients in this cluster had metastatic disease (Fig. 3). Using the broader endpoint of clinically aggressive and/or metastatic disease, the AR association in cluster 2 persisted ($p = 0.040$), whereas lower LHCGR expression in cluster 1 did not reach statistical significance ($p = 0.086$) (Fig. S5).



235

236 **Fig. 3** Hormone receptor expression in PCPG tumors by molecular cluster and presence of

237 metastasis. Within cluster 1, low LHCGR expression was associated with metastatic disease (p =

0.041). Within cluster 2, low AR expression was associated with metastasis ($p = 0.041$; two metastatic patients). Abbreviations: AR, androgen receptor; ESR1/2, estrogen receptors 1 and 2; GPER1, G protein-coupled estrogen receptor 1; LHCGR, luteinizing hormone/choriogonadotropin receptor; PCPG, pheochromocytoma and paraganglioma; PGR, progesterone receptor

In the independent E-MTAB-733 cohort, several cluster-level patterns were reproduced. As in TCGA, pseudohypoxia-related tumors had higher PGR expression (Kruskal–Wallis $p = 6 \times 10^{-14}$) and lower LHCGR expression ($p = 1.8 \times 10^{-8}$), and kinase-signaling-related tumors had the lowest AR ($p = 6 \times 10^{-4}$) and GPER1 ($p = 7.4 \times 10^{-9}$) expression. ESR1 expression was highest in pseudohypoxia-related tumors, consistent in direction with TCGA, but did not differ significantly across classes ($p = 0.2$), and ESR2 did not differ significantly ($p = 0.085$). Because the Wnt-altered-like class was smaller than the other classes, findings involving it should be interpreted cautiously (Fig. S6).

Hormone receptor expression and event-free survival

Because only five deaths occurred, including two in cluster 1, OS was not modeled. Instead, event-free survival was examined. No receptor was associated with the clinically aggressive/metastatic endpoint (15 events), either overall or within the pseudohypoxia (8 events) or Wnt-altered (6 events) clusters; the kinase-signaling cluster had a single event, precluding estimation. Similarly, no receptor was associated with the distant metastasis endpoint (10 events) (Online Resource 2, Table S1).

Differential expression and pathway enrichment in pseudohypoxia tumors

ESR1-high cluster 1 tumors (n = 30 vs. 31 ESR1-low) showed widespread transcriptional differences (3,438 genes at FDR < 0.05), with upregulation of genes involved in transcriptional

regulation, cellular signaling, and immune signaling, whereas ESR1-low tumors showed relative enrichment of genes associated with developmental and motility-related processes (Fig. 4a; Online Resource 3). AR-high tumors showed a more restricted pattern, with fewer differentially expressed genes (44 at FDR < 0.05) and smaller fold changes than ESR1 (Fig. 4b; Online Resource 4).

GSEA revealed broad pathway-level enrichment for both receptors, with absolute NES of approximately 2.0 to 2.5 among the top-ranked pathways. ESR1-high tumors were enriched for pathways related to adaptive, humoral, and B cell-mediated immunity, cell chemotaxis, and angiogenesis, whereas AR-high tumors were enriched for pathways involved in renal and vascular development (Fig. 4c, d). AR-high tumors also showed negative enrichment of mitochondrial respiratory chain, oxidative phosphorylation, and ATP synthesis pathways, indicating relative depletion of these programs compared with AR-low tumors (Fig. 4d). Differential pathway analysis (ΔNES) showed that immune processes, including antigen receptor-mediated signaling, immunoglobulin-mediated immune responses, and B cell-mediated immunity, were more strongly enriched in ESR1-high tumors, whereas cilium movement was the only top-ranked pathway relatively favoring AR-high tumors (Fig. S7).

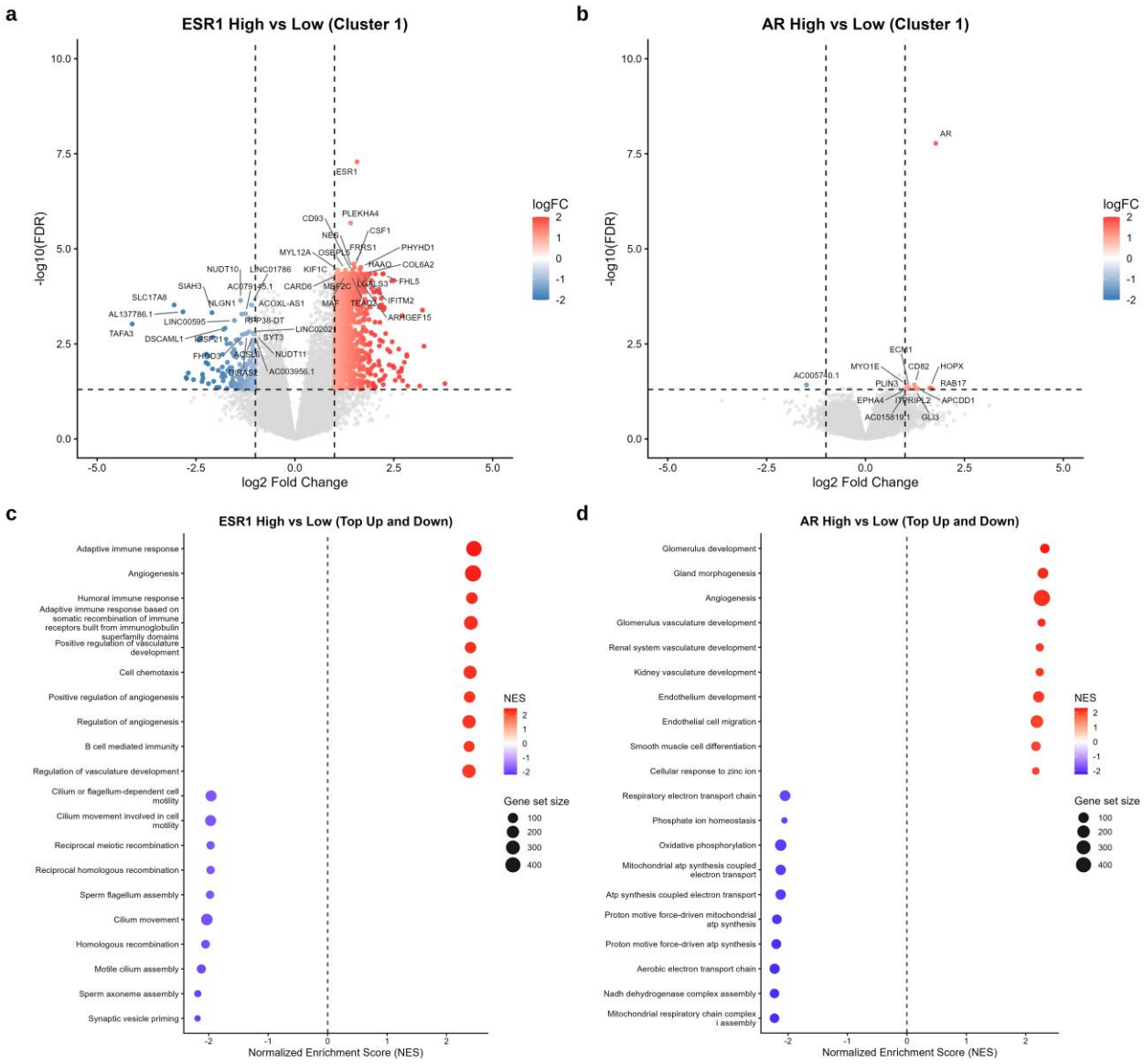


Fig. 4 Differential expression and pathway enrichment associated with ESR1 and AR in pseudohypoxia PCPG. (a) Volcano plot of differentially expressed genes in ESR1-high versus ESR1-low cluster 1 tumors (n = 61; dichotomized at the median). (b) Volcano plot of differentially expressed genes in AR-high versus AR-low cluster 1 tumors. (c) GSEA of top enriched Gene Ontology Biological Process pathways in ESR1-high tumors, showing prominent immune and inflammatory pathway enrichment. (d) GSEA of top enriched pathways in AR-high tumors, showing enrichment of renal and vascular development programs and negative

enrichment of mitochondrial electron transport and oxidative phosphorylation pathways. Abbreviations: AR, androgen receptor; ESR1, estrogen receptor alpha; GSEA, gene set enrichment analysis; NES, normalized enrichment score; PCPG, pheochromocytoma and paraganglioma

Discussion

This study represents, to our knowledge, the largest analysis of HR expression in PCPG to date and demonstrates substantial heterogeneity across molecular clusters, patient demographics, and transcriptional programs. Pseudohypoxia tumors exhibited a distinct HR profile characterized by higher ESR1 and PGR and lower LHCGR expression relative to clusters 2 and 3. The inverse associations of ESR1 and PGR with age, together with the positive association of LHCGR with age, may partly contribute to this pattern, because cluster 1 tumors are enriched for SDHx-related disease and present at younger ages. These age-associated patterns were observed predominantly in male patients and were not reproduced in PRAD, supporting the possibility that they reflect PCPG-specific biology rather than generalized age-related hormonal changes. Given that age-related variation in HR expression depends on both receptor subtype and tissue context [38–40], HR regulation in PCPG may have distinct biologic features.

Glucocorticoid receptor (NR3C1) expression did not differ across molecular clusters and was not associated with outcomes. Because cortical glucocorticoids induce epinephrine synthesis in chromaffin cells [19], GR abundance might have been expected to track with the adrenergic kinase-signaling phenotype; its uniform expression suggests that differences in local glucocorticoid exposure, for example related to adrenal versus extra-adrenal location, may be more relevant to catecholamine phenotype than receptor abundance.

Among all TCGA tumor types, PCPG demonstrates the highest LHCGR expression; within PCPG, however, cluster 1 tumors show relatively lower LHCGR expression, consistent with prior findings [18]. While hCG-mediated activation of the LH receptor has been implicated in catecholamine production and clinical symptomatology, our results suggest a novel association between higher LHCGR expression and non-metastatic disease in cluster 1 tumors, although this association did not reach significance for the broader clinically aggressive endpoint. LHCGR expression may therefore reflect a more differentiated or secretory tumor phenotype, although it was not associated with event-free survival in our cohort.

Prior in vitro studies demonstrated anti-tumor effects of estradiol, testosterone, and GPER agonism in PCPG [10]. In our cohort, we found no association between HR expression and event-free survival, and the small number of deaths precluded evaluation of overall survival. Our data therefore cannot address whether HR signaling influences outcomes in patients. Translating in vitro findings will also need to account for differences between experimental systems and human tumors, including supraphysiologic hormone exposure in vitro and the subtype-specific context of HR signaling in PCPG.

To characterize the transcriptional programs associated with ESR1 and AR, we performed differential expression and pathway analyses in cluster 1 tumors. ESR1-high tumors demonstrated widespread transcriptional activation and stronger enrichment of immune-related pathways, including antigen receptor-mediated signaling, immunoglobulin-mediated responses, and B cell-mediated immunity, whereas AR-high tumors showed a more limited transcriptional signature with enrichment of renal and vascular development pathways. One interpretation is that high ESR1 expression marks a broader, potentially dysregulated transcriptional state that includes enhanced immune and inflammatory signaling, consistent with the role of ERα as a

chromatin-dependent transcription factor with wide-ranging transcriptional output; chronic tumor-promoting inflammation has been linked to progression, invasion, and metastasis across malignancies [41, 42]. Pseudohypoxic and SDHx-mutated PCPGs display distinct immune signatures and altered inflammatory microenvironments [43], and the immune enrichment of ESR1-high tumors may intersect with these phenotypes. Within these predominantly SDHx-related pseudohypoxia tumors, AR-high cases also showed relative depletion of mitochondrial electron transport and oxidative phosphorylation pathways, raising the possibility that AR expression marks tumors with a less pronounced metabolic signature. The more restricted transcriptional profile and attenuated mitochondrial programs of AR-high tumors may be more consistent with a differentiated or regulated cellular state. These interpretations remain speculative.

HR expression was not associated with event-free survival, overall or within clusters, and the few deaths in this cohort precluded OS analysis. These null findings should be interpreted cautiously: EFS events were limited (15 aggressive/metastatic and 10 metastatic events), and heterogeneity in how aggressive disease is defined may reduce sensitivity. Whether HR expression carries prognostic information in PCPG therefore remains open and will require larger, independently annotated cohorts with more events.

This study has several limitations. First, outcome analyses were limited to a single retrospective dataset with only five deaths, which precluded OS analysis, and few EFS events, so the absence of survival associations does not exclude clinically meaningful effects. Second, molecular subtype may confound some associations: cluster 1 patients were younger, so pooled age–expression correlations may partly reflect subtype composition. Third, many receptor, subgroup, and endpoint comparisons were performed, and nominal associations based on very

small groups, such as AR and metastasis in cluster 2 (two metastatic patients), may represent false positives. Fourth, bulk RNA sequencing cannot distinguish tumor-intrinsic from stromal or immune cell expression; exclusion of cortical admixture samples only partially mitigates this. Fifth, external validation relied on harmonized published annotations and a different platform (microarray) rather than direct recreation of TCGA classes, and did not include survival data. Sixth, we did not perform protein-level validation; because mRNA and protein levels may diverge, particularly for ESR1, PGR, and AR, our findings should be confirmed by immunohistochemistry or other protein-based assays. Finally, the study is correlative, and mechanistic conclusions regarding HR signaling will require functional investigation.

In conclusion, HR expression in PCPG varies by molecular subtype, age, and sex. In pseudohypoxia tumors, low LHCGR expression was associated with metastatic disease, and ESR1-high and AR-high tumors were characterized by distinct immune-enriched and developmental transcriptional programs, respectively. No receptor was associated with event-free survival. HR profiling may help refine the biologic characterization of PCPG within existing molecular frameworks, but protein-level validation, larger outcome-annotated cohorts, and cluster-stratified functional studies are needed before these receptors can be considered biomarkers or therapeutic targets.

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Ethics approval. This study is a secondary analysis of de-identified, publicly available data from The Cancer Genome Atlas and ArrayExpress (E-MTAB-733). No new patients were recruited and no tissue was collected; ethics approval was therefore not required.

Consent to participate. Not applicable. All data were obtained from public, de-identified repositories.

Consent to publish. Not applicable.

Data availability. TCGA-PCPG and TCGA-PRAD RNA-sequencing data are available from the Genomic Data Commons (https://portal.gdc.cancer.gov/). The E-MTAB-733 dataset is available

from ArrayExpress (https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-733). Curated clinical annotations were obtained from the supplementary data of Fishbein et al. [6].

Code availability. Analysis code is available from the corresponding author upon reasonable request.

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Supplementary information

Online Resource 1: Supplementary Figs. S1–S7. Fig. S1: Age at diagnosis by molecular cluster. Fig. S2: Hormone receptor expression by molecular cluster stratified by patient sex. Fig. S3: Hormone receptor expression and age in TCGA-PRAD tumors. Fig. S4: NR3C1 (glucocorticoid receptor) expression across PCPG molecular clusters. Fig. S5: Hormone receptor expression by molecular cluster and clinically aggressive/metastatic disease. Fig. S6: External validation of hormone receptor expression in E-MTAB-733. Fig. S7: Differential pathway enrichment in ESR1-high versus AR-high pseudohypoxia PCPG

Online Resource 2: Table S1. Univariate Cox proportional hazards models of event-free survival by hormone receptor expression

Online Resource 3: Differentially expressed genes in ESR1-high versus ESR1-low pseudohypoxia tumors

Online Resource 4: Differentially expressed genes in AR-high versus AR-low pseudohypoxia tumors

Supplementary Files

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