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Abstract: Background/Objectives: Platelet-derived growth factor receptor alpha (PDGFRα) is a receptor involved in cell growth and differentiation, with unclear roles in ovarian tissues and potential interactions with potassium two pore domain channel subfamily K member 3 (KCNK3). This study aims to map PDGFRα expression across mouse tissues and to explore its co-expression with KCNK3 in the ovary. **Methods**: We visualized PDGFRα expression using RNA-seq data from the genotype-tissue expression (GTEx) BodyMAP across 54 human tissues and cap analysis of gene expression (CAGE) data for various mouse tissues. In PDGFRα^{EGFP} mice expressing EGFP in PDGFRα^+ cells, histological and fluorescence imaging were used to assess ovarian expression. Immunohistochemistry determined the co-localization of PDGFRα and KCNK3, and qPCR quantified their mRNA levels in the ovary, oviduct, and uterus. **Results**: PDGFRα showed high expression in human and mouse female reproductive tissues, particularly the ovary. In the PDGFRα^{EGFP} mouse model, PDGFRα was primarily found in the thecal layer and stromal cells, not in granulosa cells or oocytes. Immunohistochemistry indicated that $90.2 \pm 8.7\%$ of PDGFRα^+ cells also expressed KCNK3 in the ovarian stroma. qPCR revealed lower PDGFRα and KCNK3 expression in the ovary compared to the oviduct and uterus. **Conclusions**: This study shows that PDGFRα is predominantly expressed in ovarian stromal and theca cells and is highly co-localized with KCNK3, implying a novel role for PDGFRα+ cells in maintaining ionic balance and potentially influencing follicular development and ovarian physiology.

Keywords: ovary; platelet-derived growth factor receptor alpha; potassium channel subfamily K member 3; theca cells

1. Introduction

Platelet-derived growth factors (PDGFs) are dimeric proteins capable of forming homodimers (AA, BB, CC, DD) or heterodimers. PDGF-AA, PDGF-AB, and PDGF-BB interact with PDGF receptor alpha (PDGFRα), while PDGF-BB primarily binds to PDGF receptor beta (PDGFRβ) [1-3]. Specifically, PDGF-A, PDGF-B, and PDGF-C engage with PDGFRα, while PDGF-B and PDGF-D associate with PDGFRβ, initiating cellular responses by activating tyrosine kinase receptors [1,4,5]. Upon activation, PDGFRα and PDGFRβ promote cell proliferation, migration, vascular permeability, and wound healing [6,7].

The Human Protein Atlas reports the highest expression of PDGFRα mRNA in the human ovary, according to the consensus, HPA, GTEx, and FANTOM5 datasets, with the highest protein expression observed in the endometrium and ovary (as of August 26, 2024, source). Similarly, RNA-Seq cap analysis of gene expression (CAGE) from the RIKEN FANTOM5 project in mice tissue shows high expression levels in the uterus and ovary (Expression Atlas, August 26, 2024). PDGFRβ is also highly expressed in the ovary, while its expression levels are slightly lower than that of PDGFRa.

Earlier studies have identified PDGF ligands and receptors in oocytes within primordial, primary, and early-developing follicles, contributing to the maturation of primordial follicles [8,9]. PDGFs and other growth agents have been recognized as critical modulators in transitioning from primordial to primary follicles, with variations in mRNA expression levels supporting their role [10]. PDGFs act as angiogenic factors, recruiting smooth muscle cells and pericytes to stabilize and reinforce blood vessels, a process crucial for ovarian function [9,11,12]. New blood vessels are developed in the ovary, which is crucial for supplying nutrients and hormones, follicular growth, and corpus luteum development [13-15]. Consequently, disruptions in PDGF signaling may result in menstrual irregularities, amenorrhea, and infertility and potentially contribute to ovarian cancer development [16,17]. An increasing amount of evidence highlights the critical role of PDGF signaling and expression in ovarian function across variable species.

The **T**andem of P domains in a weak inward rectifying K$^+$ channel-related **A**cid-**S**ensitive **K**$^+$-1 (TASK-1/KCNK3) channel, a member of the two-pore domain K$^+$ channel, is prominently expressed in PDGFRα^+ cells in mice [18]. KCNK3 channels play a crucial role in establishing and maintaining the resting membrane potential of cells. They are susceptible to changes in oxygen levels and pH [19]. Additionally, KCNK3 channel activity is inhibited by various hormones, including norepinephrine, serotonin, substance P, and thyrotropin-releasing hormone [20]. In the testis, KCNK3 is expressed in Leydig and peritubular cells of adult mice, and PDGFRα is expressed in those cells [21]. Co-localization of PDGFRα and KCNK3 in these cells suggests that PDGFRα^+ cells may regulate intracellular calcium signaling and maintain ionic homeostasis [22].

In this study, we aimed to analyze the expression levels and patterns of PDGFRα in reproductive organs using PDGFRα^{EGFP} mice, which are genetically modified to express enhanced green fluorescent protein under the PDGFRα promotor, allowing precise visualization of PDGFRα-positive cells.

2. Materials and Methods

Transcriptomic Data Analysis of PDGFRα Expression

To analyze the tissue-specific expression of PDGFRα across various human tissues, Human BodyMap RNA-seq data were obtained from the Genotype-Tissue Expression (GTEx) project. The

dataset was accessed through the database of Genotypes and Phenotypes (dbGaP) under accession number phs000424.v8.p2. This analysis included transcriptomic data from 54 non-diseased tissues, representing nearly 1,000 individuals. Differential expression analysis focused explicitly on PDGFRα in various female tissues. For comparative analysis in mice, transcriptomic data were sourced from the Mouse Genome Informatics (MGI) database as part of the Functional Annotation of the Mammalian Genome 5 (FANTOM5) project. Cap Analysis of Gene Expression (CAGE) was utilized to determine the differential expression of PDGFRα across various female tissues in mice, providing insights into the tissue-specific expression patterns.

Animal models and housing conditions

This model was previously utilized in our study to investigate the expression pattern of PDGFRα in mouse testis, which provides a foundational basis for the current experiments [21]. For this study, we used heterozygous PDGFRα^{EGFP} male mice (B6.129S4-Pdgfra^tm11(EGFP)Sor/J, Stock No. 007669, aged 6-7 weeks) and wild-type (WT) females of the same age, both procured from Jackson Laboratory (Bar Harbor, ME, USA). The PDGFRα^{EGFP} mouse line carries an H2B-eGFP fusion gene under the control of the native *Pdgfra* promoter, resulting in fluorescence that mirrors the endogenous expression pattern of PDGFRα. Since homozygous expression of this gene leads to embryonic lethality, breeding was carried out using heterozygous PDGFRα^{EGFP} males mated with WT females to ensure the production of viable offspring for genotyping and experimental purposes. For the experiments described in this study, six 12-week-old heterozygous PDGFRα^{EGFP} females were utilized. All mice were housed under specific-pathogen-free conditions with a controlled 12-hour light-dark cycle at a temperature range of 20-24℃ and a humidity level of 50-60%. The mice had ad libitum access to food and sterile water. All animal experiments were performed according to ethical guidelines approved by the Gyeongsang National University Animal Care and Use Committee under protocol number (GNU-240527-M0112).

Hematoxylin and eosin (H&E) staining

Histological examination of ovarian tissues was conducted using H&E staining, following the protocol outlined in a previous study [23]. Ovarian tissues were fixed overnight at 4°C in 4% paraformaldehyde and then washed with 0.1 M PBS. The tissues were subsequently embedded in paraffin and sectioned into 5 μm-thick slices. The paraffin sections were air-dried on gelatin-coated slides, deparaffinized, and rinsed with tap water. The sections were stained with hematoxylin for 5 minutes, followed by staining with eosin for another 5 minutes. A graded series of ethanol (70% to 100%, each for 3 minutes) was used for dehydration, and sections were cleared with xylene. The slides were mounted using a Permount mounting medium (Fisher Chemical, Geel, Belgium). The stained sections were examined and photographed using an Olympus BX61VS microscope (Tokyo, Japan). To ensure consistency, five different sections from each sample were analyzed.

Immunohistochemistry (IHC)

Following deparaffinization, tissue sections were permeabilized with 0.1% Triton X-100 in PBS for 10 minutes at room temperature. After three washes with 1× PBS, sections were incubated for 60 minutes at room temperature in a blocking solution containing 1.5% bovine serum albumin (BSA) in 1× PBS. For KCNK3 detection, sections were incubated overnight at 4 °C with an Alexa Fluor® 405-conjugated anti-KCNK3 monoclonal antibody (1:200; Novus Biologicals, Centennial, CO, USA). After incubation, tissues were washed three times with cold 1× PBS and mounted using Gel/Mount™

(Biomeda Corp., Foster City, CA, USA). Images were captured using a confocal laser scanning microscope (Olympus). To assess co-localization of 17α-hydroxylase (CYP17A1) and PDGFRα, permeabilized ovarian sections were incubated overnight at 4 °C with an anti-CYP17A1 antibody (1:200; Santa Cruz Biotechnology, Dallas, TX, USA; sc-374244). After three 5-minute PBS washes, a fluorophore-conjugated secondary antibody (anti-mouse TEXAS Red, 1:500; Thermo Fisher Scientific, Carlsbad, CA, USA) was applied for 2 hours at room temperature. Sections were then rewashed in PBS (3 × 5 minutes), mounted with Gel/Mount™ (Biomeda Corp.) and visualized using confocal microscopy.

RNA isolation and quantitative real-time PCR

Mouse tissues were immediately frozen in liquid nitrogen upon collection and stored in a deep freezer until further use. For RNA extraction, the frozen tissues were homogenized in TRIzol reagent (Invitrogen, Thermo Fisher Scientific), and total RNA was isolated following the manufacturer's instructions. The isolated RNA was then reverse transcribed into complementary DNA (cDNA) using the RevertAid First Strand cDNA Synthesis Kit (Invitrogen), with oligo(dT) (18-mer) primers. The synthesized cDNA was used as a template for quantitative real-time PCR (qRT-PCR), performed with the amfiSure qGreen Q-PCR Master Mix (2X) (GenDEPOT, Altair, TX, USA). The specific primers used for the reactions are listed in Table 1. All qRT-PCR reactions were performed on a QIAquant 96 2plex system (Qiagen, Hilden, Germany). The relative expression levels of PDGFRα or KCNK3 were normalized to GAPDH and calculated using the $2^{-\Delta\Delta Ct}$ method to determine fold changes.

Table 1. Primer sequences used for qPCR.

Gene Name	Species	GenBank Accession Numbers	Primer Sequences (5'–3')	Expected size (bp)
Pdgfra	Mouse	BC053036.1	F: TGCGGGTGGACTCTGATAATGC R: GTGGAACTACTGGAACCTGTCTCG	235
KCNK3	Mouse	NM_001083316.2	F: TCCTTCTACTTCGCCATCACC R: AGGCTCTGGAACATGACTAGTGT	137
Gapdh	Mouse	GU214026.1	F: AC CAGAAGACTGTGGATGG R: CACATTGGGGGTAGGAACAC	171

Statistical analysis

Data are expressed as the **mean ± standard deviation (S.D.).** Group differences were evaluated using one-way ANOVA followed by post hoc Bonferroni correction, performed with **OriginPro 2020** software (OriginLab Corp., MA, USA). A **p-value < 0.01** was considered statistically significant.

3. Results

3.1. Dynamic expression patterns of PDGFRα in human and mouse tissues

To explore the expression patterns of PDGFRα across various human tissues, we visualized RNA-seq transcriptomic data from the GTEx BodyMAP obtained from the GTEx database. Using

RNA-seq quantification tools available on the GTEx portal, we generated expression profiles of PDGFRα from a dataset comprising 1,000 human samples across 54 different organs and tissue types, such as female reproductive tissues, spleen, adipose tissue, respiratory system, gastrointestinal tract, kidney, urinary bladder, muscle tissues, proximal digestive tract, skin, pancreas, endocrine tissues, brain, male reproductive tissues, liver, and eyes (Figure 1A). Gene expression levels are quantified as transcripts per million (TPM). We specifically visualized PDGFRα expression in female reproductive tissues, including the ovary, endocervix, fallopian tube, endometrium of the uterus, ectocervix, vagina, and breast. The human ovary exhibited the highest normalized TPM (nTPM) levels (Figure 1B).

Similarly, we visualized Cap Analysis of Gene Expression (CAGE) data for PDGFRα expression across a range of mouse tissues from public datasets. These tissues included the uterus, urinary bladder, skin, ovary, vagina, adrenal gland, aorta, axillary lymph node, corpus striatum, epididymis, prostate gland, spinal cord, tongue, lung, cerebellum, cerebral cortex, corpora quadrigemina, diencephalon, eyeball (camera-type eye), hippocampus, medulla oblongata, stomach, olfactory brain, submandibular gland, spleen, intestine, colon, and vesicular gland (Figure 1C). These data indicate that PDGFRα expression is predominantly higher in female tissues, particularly in the ovary, compared to other tissue types in humans and mice. Moreover, expression levels in human reproductive organs were notably higher than in corresponding mouse tissues, such as the ovary and uterus.
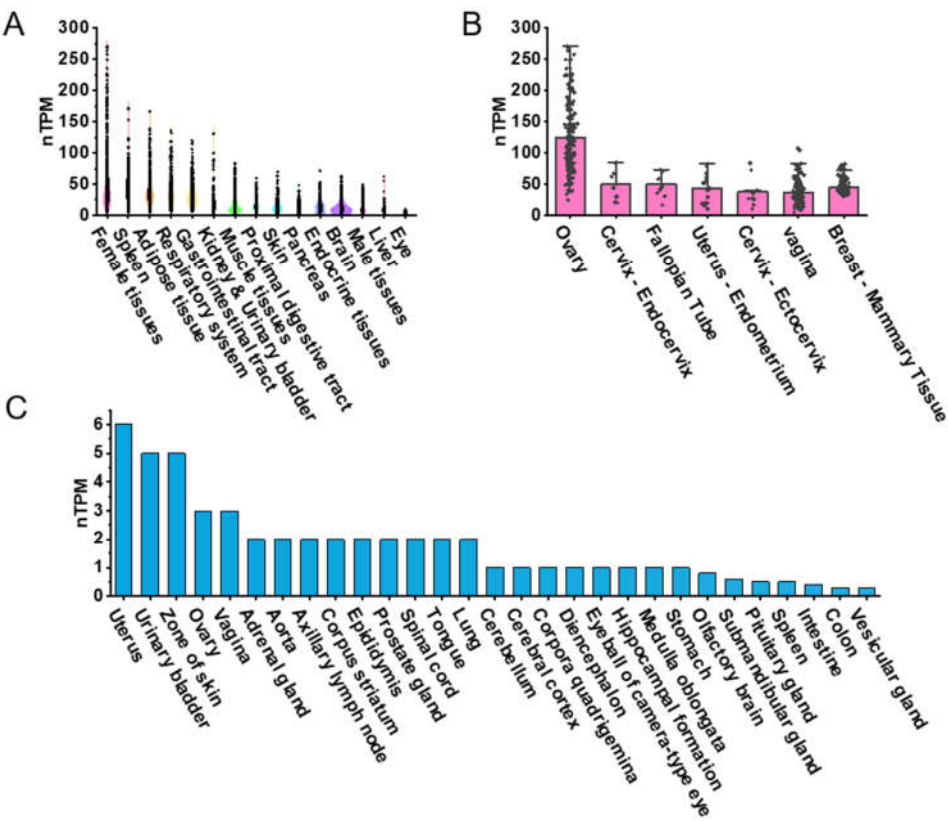

Figure 1. PDGFRα expression in human and mouse tissues based on public datasets.
(A) Violin plot highlighting the top 15 tissue types with the highest PDGFRα expression levels in humans. (B) PDGFRα expression in seven female tissue types in humans. (C) Bar graph representing PDGFRα expression across 29 different tissue types in mice.

Expression pattern of PDGFRα in PDGFRα^{EGFP} mouse ovary

In the ovaries obtained from the PDGFRα^{EGFP} mouse model, H&E staining revealed typical ovarian histology, including the presence of **secondary follicles.** Secondary follicles are characterized by multiple layers of **granulosa cells** and the **cumulus oophorus (CO)** surrounding the oocyte. Small fluid-filled spaces, indicating early antral formation, were also observed within these follicles. The **theca interna** around the granulosa cell layer was clearly visible, with no detectable pathological changes (Figure 2A, n = 3).

The PDGFRα^{EGFP} mouse model is engineered to express **EGFP** in the nuclei of **PDGFRα+ cells**, allowing for the visualization of PDGFRα expression in various tissues. In this model, the EGFP signal indicates the presence of PDGFRα^+ cells [18]. The fluorescence images (green) highlight the **EGFP expression** in the nuclei of **PDGFRα+ cells**. **PDGFRα expression** is predominantly observed in the **thecal layer (TL)** surrounding the granulosa cells in the stroma throughout the **ovarian cortex**. There is an absence of fluorescence in **granulosa cells** and **oocytes (Figure 2B).**
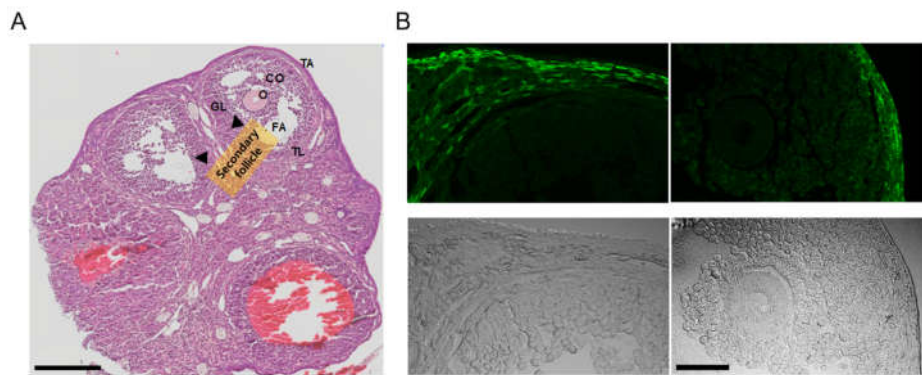

Figure 2. Analysis of ovarian tissue sections from PDGFRα^{EGFP} mice.
(A) Hematoxylin and Eosin (H&E) stained image displaying the overall ovarian histology, with clearly identified secondary follicles (indicated by arrows). Granulosa cells (GL) surround the oocyte (O), along with the cumulus oophorus (CO). The theca layers (TL) surrounding the follicles are visible. FA represents the follicular antrum, and TA represents the tunica albuginea. NC represents the negative control, where no anti-KCNK3 antibody treatment was applied. Scale bar, 200 μm. (B) Fluorescent imaging of ovarian tissues from PDGFRα^{EGFP} mice. The top panels show fluorescence imaging, and the bottom panels show corresponding brightfield images. Scale bar, 50 μm.

Co-localization of PDGFRα and KCNK3 in the ovary

Immunohistochemical analysis showed that KCNK3 is expressed in PDGFRα^+ cells within the ovarian stroma (Figure 3A). In the negative control (NC), where the anti-KCNK3 antibody was omitted, only PDGFRα expression was observed in the stroma without KCNK3 expression (Figure 3A). Quantitative analysis showed that 90.2 **± 8.7% of** PDGFRα^+ cells also expressed KCNK3**, indicating that most KCNK3$^+$ cells are PDGFRα^+ cells (Figure 3B, n = 4).**

To determine the **mRNA levels of PDGFRα** in the ovary, **quantitative PCR (qPCR)** analysis was conducted and compared with the oviduct and uterus. The qPCR results indicated that **PDGFRα expression** in the ovary was lower than in both the oviduct and uterus. There was no significant difference in **PDGFRα expression** levels between the oviduct and uterus. Like PDGFRα, KCNK3 expression levels were lower in the ovary than in the oviduct and uterus (Figure 3C, p < 0.01). As

shown in Figure 3D, PDGFRα was found to co-localize with 17α-hydroxylase (CYP17A1), a well-established marker of theca cells, in the thecal layer, supporting the presence of PDGFRα^+ cells in this region. This finding confirms that a subset of theca cells expresses PDGFRα, suggesting its potential involvement in the steroidogenic function of these cells. The negative control (NC) showed no detectable non-specific staining, confirming the specificity of the immunostaining.
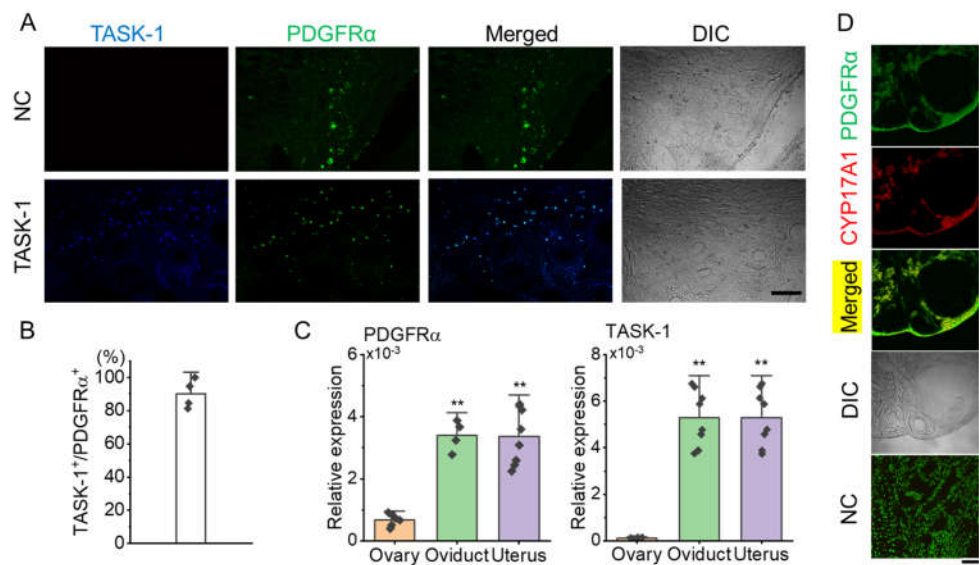

Figure 3. Co-localization of KCNK3 and PDGFRα in ovarian tissue sections from PDGFRα^{EGFP} mice. (A) Co-localization of PDGFRα (green) and KCNK3 (blue) in ovarian tissues. Merged and differential interference contrast (DIC) images are shown to visualize cell structure. Scale bar, 50 µm. (B) Bar graph showing the percentage of KCNK3 co-localization with PDGFRα^+ cells. (C) PDGFRα and KCNK3 transcript expression levels in mice's ovary, oviduct, and uterus. Each bar represents the mean ± S.D. for four to eight samples. **p < 0.01 compared to the ovary. (D) Co-localization of PDGFRα and CYP17A1 in the thecal layer. PDGFRα (green) is expressed in ovarian stromal and thecal cells, while CYP17A1 (red) marks theca cells explicitly. Merged images indicate partial co-localization of PDGFRα and CYP17A1. DIC: Differential interference contrast image. NC: Negative control. Scale bar, 50 µm.

4. Discussion

This study analyzes **PDGFRα** expression patterns across various human and mouse tissues using publicly available datasets. RNA expression data from the Human Protein Atlas reveal distinct tissue-specific expression profiles for **PDGFRα**, with a predominant presence in female reproductive tissues, particularly in the ovary. Similarly, **CAGE** data for mice show that **PDGFRα** is predominantly expressed in female tissues, particularly in the uterus and ovary. However, the expression levels of **PDGFRα** in human reproductive organs, such as the ovary and uterus, were markedly higher than those observed in corresponding mouse tissues. These differences may reflect species-specific regulatory mechanisms and underscore the need for careful cross-species comparisons when interpreting gene expression data.

This study aimed to investigate the expression of PDGFRα in the mouse ovary using PDGFRαEGFP mice to assess whether PDGFRα is highly expressed in the ovary, though at lower levels compared to the human ovary. Histological analysis of the PDGFRαEGFP mouse model revealed a typical ovarian architecture indicative of normal ovarian development. Fluorescence

imaging further demonstrated that PDGFRα expression was localized in ovarian cortex stromal cells, including the thecal layers. The lack of PDGFRα expression in granulosa cells and oocytes suggests that PDGFRα has a more specific role in the ovarian stromal compartment, particularly in the thecal cells, which are involved in steroidogenesis and follicle support. Previous studies have shown that PDGFRα is a gene specifically enriched in theca cells [24,25].

Theca cells play a critical role in folliculogenesis, contributing to several essential processes. They are primarily responsible for synthesizing androgens, which are converted into estrogens by granulosa cells, providing the hormonal support necessary for follicle development [26]. Theca cells also engage in crosstalk with granulosa cells and oocytes, facilitating communication and coordination during follicular growth. Additionally, they offer structural support to the growing follicle as it progresses through its developmental stages toward producing a mature, fertilizable oocyte. Theca cells are fundamental for follicular growth by supplying all the androgens needed for estrogen production, a key factor for follicle maturation. In follicles that successfully ovulate, theca cells undergo hormone-dependent differentiation to become luteinized thecal cells in the corpus luteum, contributing to progesterone production necessary for maintaining early pregnancy [26].

Ovarian stem/progenitor cells, crucial for folliculogenesis and tissue repair following ovulation, express PDGFRα. PDGFRα^+ cells are specifically located in the interstitial compartment. LY6A$^+$ and PDGFRα^+ cells differentiate into steroidogenic cells expressing CYP11A1 and CYP17A1, primarily producing androgens and exhibiting characteristics similar to theca cells [27]. Our previous study observed PDGFRα expression in Leydig cells but not in germ or Sertoli cells [21]. These findings suggest that PDGFRα is closely linked to regulating androgenic hormone production, as Leydig and theca cells are key producers of testosterone.

KCNK3 channels, which co-localize with PDGFRα in Leydig cells, are also present in ovarian stromal cells, including theca cells, indicating a role for KCNK3 in androgen hormone production. Our previous study showed that KCNK3 is highly expressed in Leydig cells compared to Sertoli cells. In Leydig cells, blocking KCNK3 led to apoptosis, suggesting that KCNK3 activation is linked to cell proliferation [22]. KCNK3 is also the most prominently expressed K$^+$ channel in mouse jejunal PDGFRα^+ cells [18], exhibiting more hyperpolarized membrane potentials than smooth muscle cells in the gastrointestinal tract [28]. This suggests that KCNK3 channels may contribute to maintaining hyperpolarized membrane potentials in PDGFRα^+ cells. The regulation of membrane potential and cell proliferation through KCNK3 is likely associated with androgen hormone regulation in PDGFRα^+ cells, such as theca cells. Additionally, the co-expression of **PDGFRα** and **KCNK3** in ovarian stromal cells suggests potential functional interactions between these two proteins in ovarian physiology.

Quantitative PCR analysis revealed that the expression levels of PDGFRα and KCNK3 were lower in the ovary than in the oviduct and uterus. This differential expression pattern suggests that while PDGFRα and KCNK3 play essential roles in the ovary, their functions may be more pronounced or have distinct regulatory roles in other reproductive tissues, such as the oviduct and uterus. The similar expression trends of PDGFRα and KCNK3 across these tissues support the idea of a coordinated regulatory mechanism that may be crucial for maintaining tissue-specific physiological processes, particularly in the female reproductive system. PDGFRα is likely expressed in fibroblasts and smooth muscle cells within the stroma of the fallopian tubes. It is also expected to be expressed in stromal cells, fibroblasts, and uterine smooth muscle cells in the uterus. This expression pattern may account for the higher levels of PDGFRα in the fallopian tubes and uterus compared to the ovaries. PDGFRα plays a crucial role in cell proliferation, tissue regeneration, and

angiogenesis [29,30], suggesting that its expression may be significantly elevated under proliferative or pathological conditions of the endometrium. Additionally, KCNK3 could serve as a marker for PDGFRα+ cells and may also be involved in modulating the known functions of PDGFRα.

5. Conclusions

In conclusion, this study highlights the distinct expression patterns of PDGFRα across human and mouse tissues, with the ovary showing high expression in both species, although more pronounced in humans. In the PDGFRα^EGFP mouse model, PDGFRα was localized primarily to thecal cells in the ovarian stroma, suggesting a role in steroidogenesis and follicular development. Additionally, significant co-localization of PDGFRα and KCNK3 in the ovary points to potential functional interactions in regulating reproductive physiology. These findings suggest that PDGFRα and KCNK3 play critical roles in ovarian function and may be involved in broader reproductive processes.

Author Contributions: Conceptualization, E.-A.K. and D.K.; methodology, S.H., C.-W.K., I.A.C., J.K.S., D.K.L., D.K.; software, B.A. and D.K.; validation, D.-K.L. and D.K.; formal analysis, S.K. and D.K.; investigation, B.A., E.-J.K., J.-M.K. and D.K.; data curation, D.K.; writing—original draft preparation, E.-A.K. and D.K.; writing—review and editing, D.K.; visualization, B.A. and D.K.; supervision, D.-K.L. and D.K.; project administration, E.-J.K.; funding acquisition, D.K. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement: The animal study was approved by the Institutional Ethics Committee of Gyeongsang National University (protocol code GNU-240527-M0112 and 2024.05.27).

Data Availability Statement: Publicly available transcriptomic datasets used in this study were obtained from the Genotype-Tissue Expression (GTEx) project (https://gtexportal.org/home/) and the FANTOM5 Cap Analysis of Gene Expression (CAGE) dataset (https://fantom.gsc.riken.jp/5/). The accession number for the GTEx data is phs000424.v8.p2. Additional experimental data generated during this study (e.g., qPCR, immunostaining) are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

KCNK3	potassium two pore domain channel subfamily K member 3
PDGFRα	Platelet-derived growth factor receptor alpha
TASK-1	**T**andem of P domains in a weak inward rectifying K$^+$ channel-related **A**cid-**S**ensitive **K**$^+$-1

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