Pang Q et al. (2024) INVESTIGATING PIGMENT EPITHELIUM-DERIVED FACTOR EXPRESSION AND ITS DISTRIBUTION IN SHEEP TISSUES: IMPLICATIONS FOR SPORTS MEDICINE. Revista Internacional de Medicina y Ciencias de la Actividad Física y el Deporte vol. 24 (96) pp. 568-584.
DOI: https://doi.org/10.15366/rimcafd2024.96.035

ORIGINAL

INVESTIGATING PIGMENT EPITHELIUM-DERIVED FACTOR EXPRESSION AND ITS DISTRIBUTION IN SHEEP TISSUES: IMPLICATIONS FOR SPORTS MEDICINE

Rui Liang [1], Zhen Li [2], Xiaorui Fan [1], Quanhai Pang [1,*]

[1] College of Veterinary Medicine, Shanxi Agricultural University, Taigu, Shanxi, 030801, PR China
[2] College of Basic Medical Sciences, Shanxi University of Chinese Medicine, Jinzhong, 030619, PR China
E-mail: pangquanhai@163.com

Recibido 22 de octubre de 2023 **Received** October 22, 2023
Aceptado 22 de puede de 2024 **Accepted** May 22, 2024

ABSTRACT

Objective: Pigment epithelium-derived factor (PEDF) plays a crucial role in various biological functions such as anti-angiogenesis, neuroprotection, and tumor suppression. Despite its importance, the expression and distribution of PEDF in normal mammalian tissues remain inadequately understood. This study aims to elucidate the expression levels and localization of PEDF across different tissues in healthy sheep, providing insights that may benefit regenerative medicine in sports. **Methods:** Three healthy sheep were utilized to assess the PEDF expression in diverse tissues including the heart, liver, spleen, lungs, kidneys, cerebrum, cerebellum, caput epididymides, corpora epididymides, cauda epididymides, and testes. Following euthanasia, these tissues were harvested for analysis. The expression and localization of PEDF were examined through quantitative real-time PCR (qRT-PCR), western blotting, and immunohistochemistry. **Results:** PEDF was variably expressed in all examined tissues, displaying a consistent trend at both mRNA and protein levels. Visceral tissues showed significantly higher PEDF expression in the lungs compared to the heart, liver, spleen, and kidneys ($p < 0.01$). In brain tissues, PEDF levels were notably higher in the cerebrum than in the cerebellum. Among gonadal tissues, the highest expression was observed in the caput epididymis, with similar levels in the corpora epididymides, cauda epididymides, and testes. Immunohistochemical staining confirmed PEDF presence in the connective tissue between cardiomyocytes, visceral tissue cell

cytoplasm, brain tissue nerve fibers, and the cytoplasm of testicular and epididymal epithelial cells. **Conclusion:** The widespread expression of PEDF mRNA and protein across sheep tissues highlights its potential multifunctional role. The variation in expression levels and locations suggests that PEDF's biological functions are closely linked to its distribution. This study lays the groundwork for future investigations into the therapeutic applications of PEDF, particularly in the field of sports medicine, where its regenerative and protective properties could be harnessed to improve athlete recovery and performance.

KEYWORDS: PEDF; Tissues; Gene Expression; Sheep

1. INTRODUCTION

Pigment Epithelium-Derived Factor (PEDF) is a multifunctional protein belonging to the non-inhibitory serpin family, which has garnered considerable attention in biomedical research due to its diverse biological activities, including anti-angiogenesis, neuroprotection, and anti-tumor effects. Originally identified in the retina, PEDF is now known to be widely expressed across various tissues and has significant implications for both pathological and physiological processes. Despite its well-documented roles in various human tissues, comprehensive data on the distribution and expression levels of PEDF in normal physiological conditions, particularly in animal models such as sheep, remain sparse (Cao et al., 1999; Pak et al., 2004). This gap in knowledge is significant given the potential of PEDF in therapeutic applications, including regenerative medicine and sports medicine, where its properties could notably enhance recovery and overall health outcomes in athletes. Sheep serve as valuable models for human physiology due to similarities in size and organ function, making them particularly useful for studies on tissue regeneration and injury recovery (Carrell et al., 1991; Travis & Salvesen, 1983; Zhou et al., 2006). Understanding the natural expression patterns of PEDF in sheep tissues could provide critical insights into its potential roles in tissue repair and maintenance, which are central to sports medicine. The objective of this study is to systematically analyze the expression and localization of PEDF across various sheep tissues (Tombran-Tink et al., 1994; Wu & Becerra, 1996). This research involves detailed quantification of PEDF using state-of-the-art molecular techniques, including quantitative real-time PCR (qRT-PCR), western blotting, and immunohistochemistry. By mapping the distribution of PEDF and examining its concentration in different tissues, this study aims to shed light on the potential physiological roles of PEDF and set a foundation for future therapeutic strategies that could mitigate injury and accelerate recovery in athletic settings. This investigation is poised to expand our understanding of PEDF's functional dynamics in vivo, highlighting its relevance not only in normal physiology but also in the context of sports-related injuries and recovery processes. Such insights are expected to catalyze further research into PEDF's therapeutic potentials, particularly in orthopedics and muscle regeneration,

areas of immense interest in sports medicine (Perez-Mediavilla et al., 1998; Tombran-Tink & Johnson, 1989; Wu et al., 1995).

2. Materials and Methods

2.1. Animals and samples

Three 12-month-old male sheep with mean weight of 50 ± 0.5 kg were fed *ad libitum*. The Animal Ethics Committee of Shanxi Agricultural University (Taigu, Shanxi, China) authorized the euthanasia of the sheep, which was done in accordance with the International Guiding Principles for Biomedical Research Involving Animals. The collected specimens included the heart, liver, spleen, lungs, kidneys, cerebrum, cerebellum, caput epididymides, corpora epididymides, cauda epididymides and testes. These samples were stored at -80℃ and then pulverized in liquid nitrogen.

2.2. Detection of mRNA abundance of PEDF in visceral tissues of sheep

Primers were designed and synthesized based on reference sequences retrieved from the NCBI database. The sequences of primer for PEDF were as follows:

Forward 5′ - AGACATCCACGGCACCTACA -3′

Reverse 5′ - TATCCGCAGCTTCCTCTCAA -3′

The expected amplicon size of the PEDF fragment was 103 bp. Total RNA was extracted from different tissues of sheep as per the manufacturer's instructions providing with the Total RNA Isolation Kit (Sangon Biotech, Shanghai, China). Similarly, cDNA was synthesized as per the manufacturer's instructions providing with the reverse transcription kit (TransGen Biotech, Beijing, China). Reverse transcription reaction mixture (20 μL) included Total RNA 500 ng, Random Primer 1 μL, 2×ES Reaction Mix 10 μL, EasyScript® RT/RI Enzyme Mix 1 μL, and gDNA Remover 1 μL. After refilling with RNase-free Water to 20 μL, the mixture was incubated at 25℃ for 10 min, 42℃ for 15 min, and then inactivated at 85℃ for 5 s. Conventional PCR amplification was performed using the primers combined with the cDNA from different sheep tissues as the templates. The PCR amplicons were purified and recovered for use as target genes, ligated using the pEASY-T1 plasmid vector (TransGen), and transformed into DH5α competent *Escherichia coli* cells. Positive clones were selected and cultured, and the recombinant plasmid DNA was extracted. The DNA concentration in eight 10-fold serially diluted solutions was measured using a spectrophotometer, and a standard curve was plotted. Finally, qPCR was performed as per the instructions provided with the SYBR Green PCR reagent kit (TransGen Biotech), and absolute quantitative standard curves were plotted. Next, fluorescence qPCR was performed using the cDNAs from

different tissues of sheep as templates. The reaction mixture (20 μL) included 10 μL qPCR Mix, 0.5 μL forward primer (10 μM) and 0.5 μL reverse primer (10 μM) for the appropriate target, 2.0 μL cDNA template, and 7.0 μL nuclease-free ddH_2O. The PCR thermal profile included a 94℃-holding step for 30 s, followed by 40 cycles of 94℃ for 5 s, 61℃ for 35 s, and 97℃ for 10 s. All experiments were performed with six replicates, and the absolute abundance of PEDF mRNA in different visceral tissues was automatically calculated using the absolute quantification standard curve.

2.3. Determination of PEDF expression at protein level

RIPA buffer (Beyotime Biotechnology, Shanghai, China) (1 mL) was added to the sample of different tissues for total protein extraction. The protein concentration was measured using a BCA assay kit (Solarbio, Beijing, China). Target proteins were prepared for SDS-PAGE gel electrophoresis, and the separated proteins were transferred to a PVDF membrane through the wet transfer method at a constant current of 300 mA for 30 min in an ice bath. The membrane was blocked by incubating it with blocking solution for 2 h, with gentle shaking. Next, the anti-PEDF primary antibody (Bioss, Beijing, China, bs-20784R, 1:1000 dilution) and anti-GAPDH internal reference antibody (Solarbio, K106389P, 1:3000 dilution) were added and the solution was incubated overnight at 4℃, followed by repeated washing with TBST. Next, sheep anti-rabbit IgG-HRP (Solarbio, SE134, 1:3000 dilution) was added and the mixture was incubated at 37℃ for 1 h, and then washed five times with TBST. Finally, the ECL color development substrate solution (Solarbio) was added and the membranes were scanned using a ChemiDOC XRS+Imager (Bio-Rad Laboratories). The levels of PEDF and GAPDH proteins were quantified using Image-Pro Plus Software (Olympus, Tokyo, Japan).

2.4. Immunohistochemical localization of PEDF in different tissues of sheep

Paraffin-embedded, paraformaldehyde-fixed tissues were cut into 4 μm sections using a microtome, and the sections were baked overnight at 58℃. The sections were deparaffinized and dehydrated in citric acid solutions (pH 6.0). Antigen retrieval was performed through microwave heating. The sections were cooled to 25℃, and then removed and immersed in 3.0% H_2O_2 solution for 20 min to remove endogenous peroxidase from the sectioned tissues. Next, the sections were washed with PBS and blocked with 5% BSA solution for 20 min. Anti-PEDF primary antibody (1:1000 dilution) was added and incubated overnight at 4℃. Then, the sections were washed with PBS, sheep anti-rabbit IgG-HRP secondary antibody (1:3000 dilution) was added, and the mixture was incubated for 30 min at 37℃. Finally, the sections were washed thrice with PBS, incubated with DAB for 5 min, counterstained with hematoxylin, rinsed with ddH_2O, dehydrated through an alcohol gradient, and mounted with neutral gum.

The slides were observed using an Olympus microscope (Leica Microsystems, Buffalo Grove, IL, USA) and imaged. For negative control samples, the primary antibody solution was replaced with PBS. The images were acquired and analyzed using the Image-Pro Plus Software (Olympus, Tokyo, Japan).

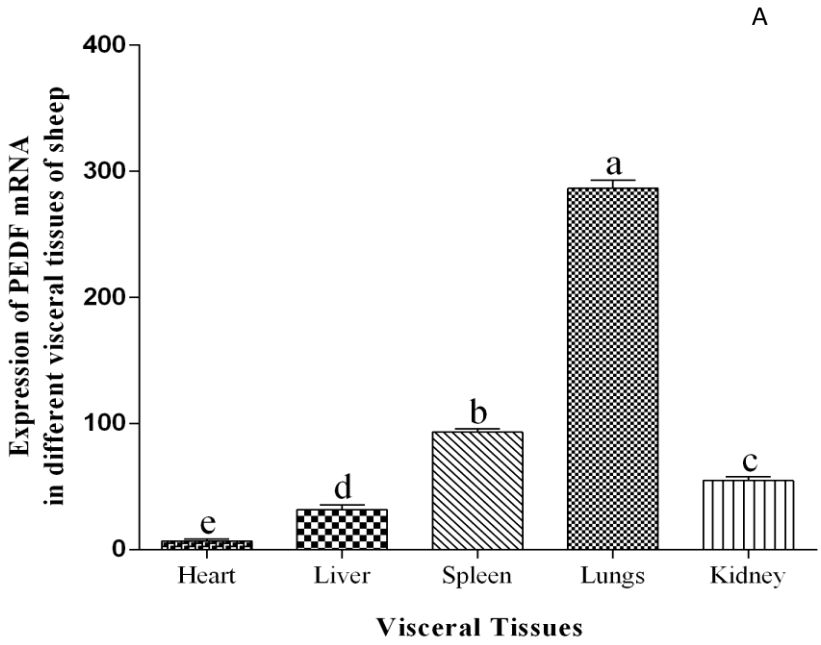
2.5 Statistical analysis

The SPSS 17.0 software was used for data analysis. The paired t-test was used to analyze the differences in the results. The results were presented as mean ± SD. The differences with $p < 0.05$ were considered significant, while the differences with $p < 0.01$ were considered highly significant.

3. Results

3.1 Expression of PEDF gene in different tissues of sheep

The absolute quantification results revealed that PEDF mRNA was expressed in all sheep tissues (Figure 1). In visceral tissues (Figure 1A), the lung had the highest mRNA abundance of PEDF, followed by spleen, kidney and liver, and the lowest value in heart, with highly significant differences among different tissues ($p < 0.01$). In the brain and gonadal tissues of sheep (Figure 1B), the mRNA abundance of PEDF was significantly higher ($p < 0.01$) in the caput epididymis than in the brain and other gonadal tissues. The expression in the corpora epididymides and testes was the second highest, and the mRNA abundance of PEDF in the brain was not different ($p < 0.01$) from that in the cauda epididymis but was lower than that in the corpora epididymis and testes. The mRNA abundance of PEDF was the lowest in the cerebellum ($p < 0.01$).



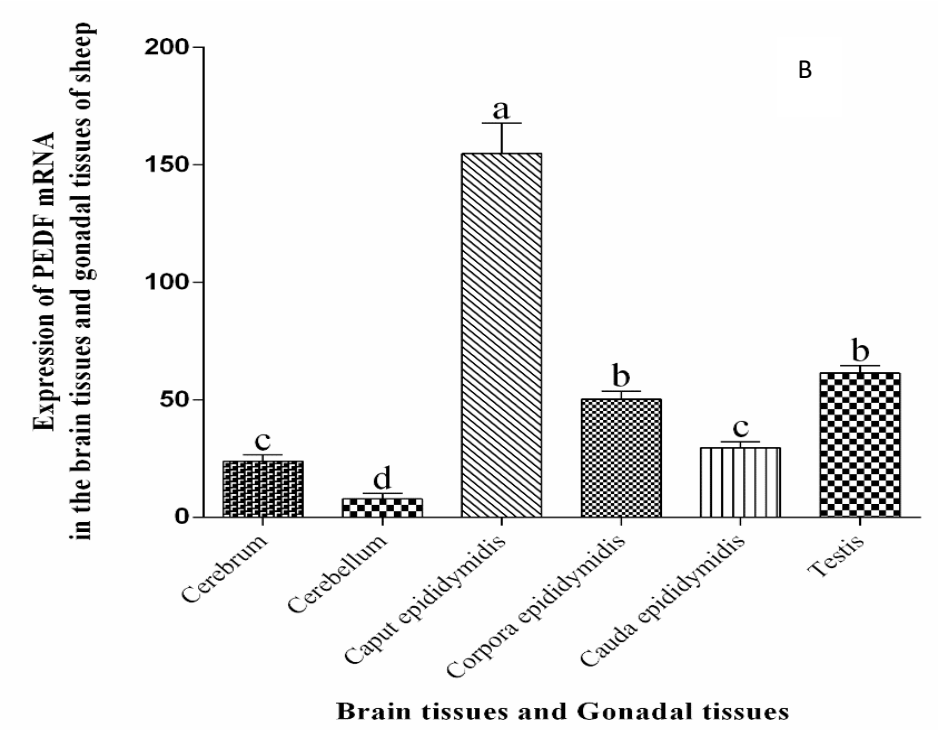
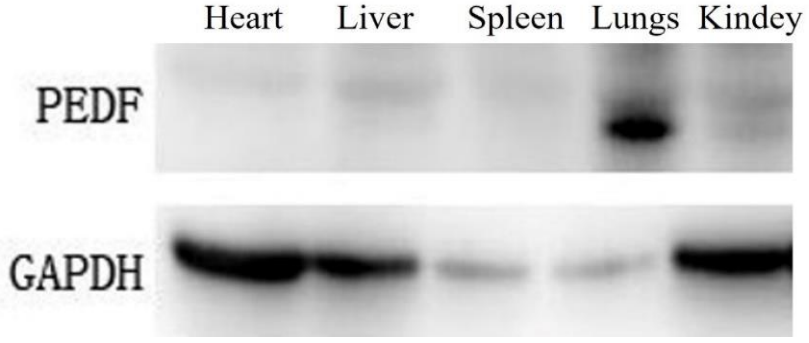


Figure 1: The mRNA abundance of pigment epithelium-derived factor (PEDF) in the different visceral tissues (A), and brain and gonadal tissues of sheep (B). a, b, c, d, e: Means with different superscripts differ ($p < 0.01$).

The total protein bands were clear (Figures 2 and 3) and suitable for the determination of PEDF protein levels. The PEDF was expressed in all tissues of sheep, with significantly different expression levels ($p < 0.01$) among the different tissues. In visceral tissues (Figure 2), The PEDF protein expression was the highest in lung, followed by spleen, kidney and liver, with the lowest value in heart ($p < 0.01$).

In the brain and gonadal tissues of sheep (Figure 3), the PEDF protein expression was significantly higher ($p < 0.01$) in the caput epididymis than in the brain and other gonadal tissues. The PEDF expression was not different among other gonadal tissues ($p > 0.01$) and between the cerebrum and cerebellum ($p > 0.01$). The results were consistent with those obtained at mRNA abundance.



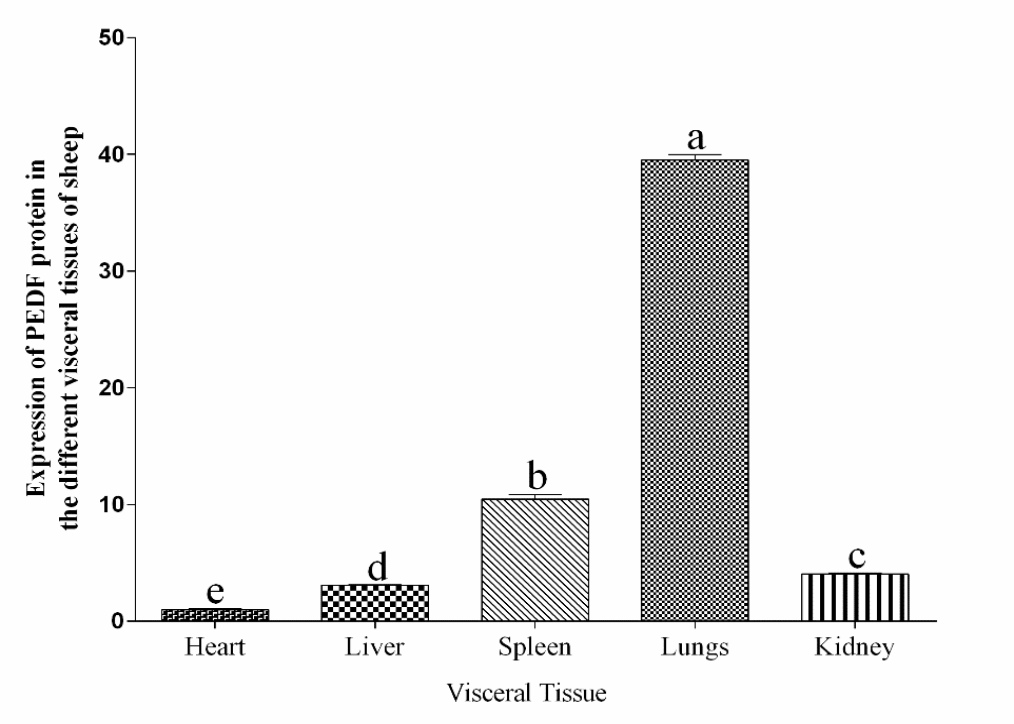


Figure 2: Western blot analysis for pigment epithelium-derived factor (PEDF) protein in the different visceral tissues of sheep. GAPDH was used as an internal control. a, b, c, d, e: Means with different superscripts differ ($p < 0.01$).

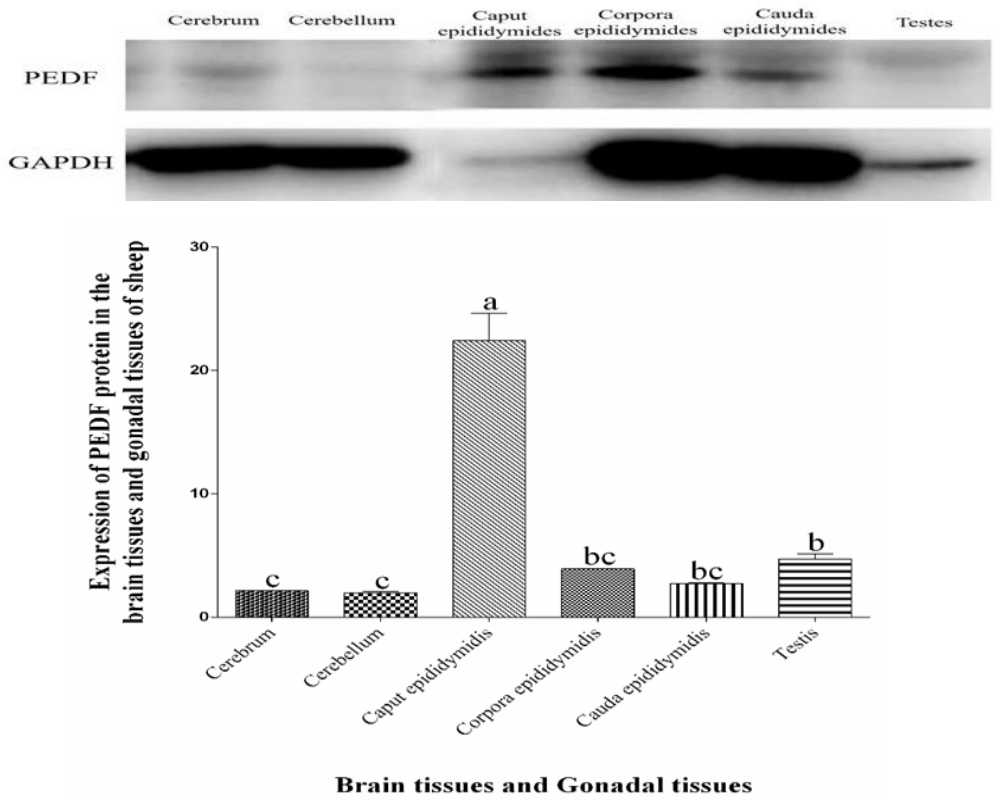


Figure 3: Western blot analysis for pigment epithelium-derived factor (PEDF) protein in the different brain tissues and gonadal tissues of sheep. GAPDH was used as an internal control. a, b, c: Means with different superscripts differ ($p < 0.01$).

3.2 Immunohistochemical localization of PEDF in different visceral tissues of sheep

The PEDF protein staining exhibited a blue-purple color in control sheep tissue sections and brown in experimental tissue sections (Figures 4, 5 and 6). In visceral tissues (Figure 4), PEDF protein localized in the connective tissues between cardiomyocytes (Figure 4A), the cytoplasm of macrophage in the hepatic sinuses (Figures 4B); weakly expressed in cytoplasm of hepatocytes (Figures 4B) and splenic lymphocyte (Figures 4C), the cytoplasm of alveolar macrophages (Figure 4D), and cytoplasm of renal tubular epithelial cells (Figure 4E). In brain tissues (Figure 5), PEDF protein is localized in the nerve fibers around the vertebral cells of the cerebral cortex (Figure 5A), nerve fibers around cerebellar granulosa cells (Figure 5B), nerve fibers in the cerebellar medulla (Figure 5B). In gonadal tissues (Figure 6), PEDF protein localized in the epithelial cell cytoplasm of the caput epididymides, corpora epididymides and cauda epididymides (Figure 6A, Figure 6B, Figure 6C) and the rounded sperm cell cytoplasm，sperm cells, primary spermatocyte, spermatogonia in testes (Figure 6D).

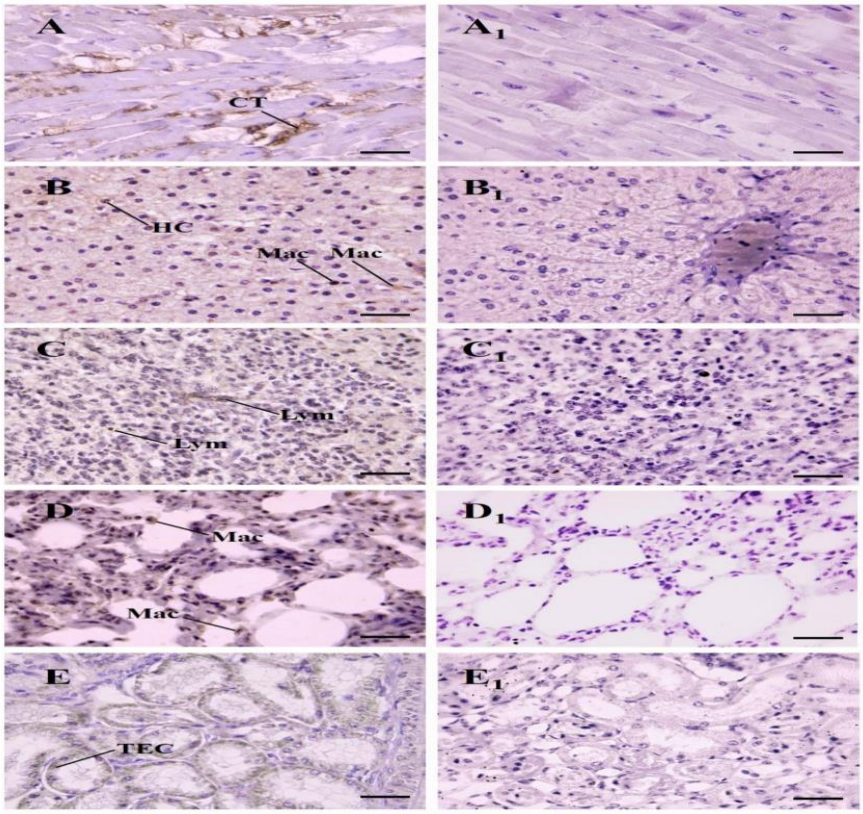


Figure 4: Immunohistochemical staining of pigment epithelium-derived factor in sheep visceral tissues. The images in the left side with no subscript indicates the experimental group; and those in the right side with subscript 1 indicates the respective negative control. A, heart; B, liver; C, spleen; D, lung; E, kidney. CT, connective tissue; HC, hepatocyte; Mac, Macrophages; Lym, Lymphocytes; TEC, renal tubular epithelial cell. Bar = 25 μm.

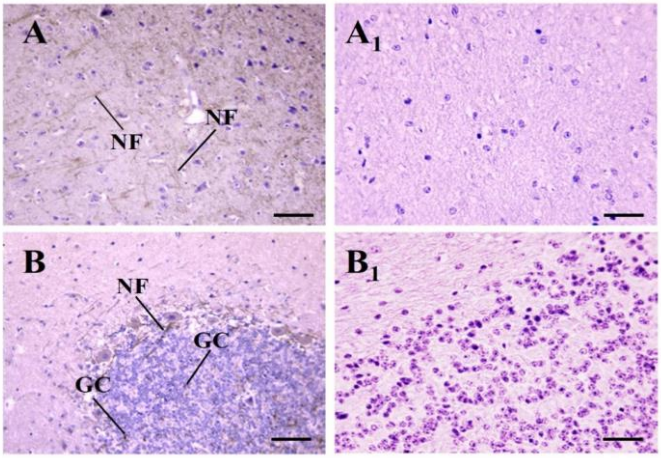


Figure 5: Immunohistochemical staining of pigment epithelium-derived factor in sheep brain tissues. The images in the left side with no subscript indicates the experimental group; and those in the right side with subscript 1 indicates the respective negative control. A, cerebrum; B, cerebellum. NF, nerve fiber; GC, granular cell. Bar = 25 μm.

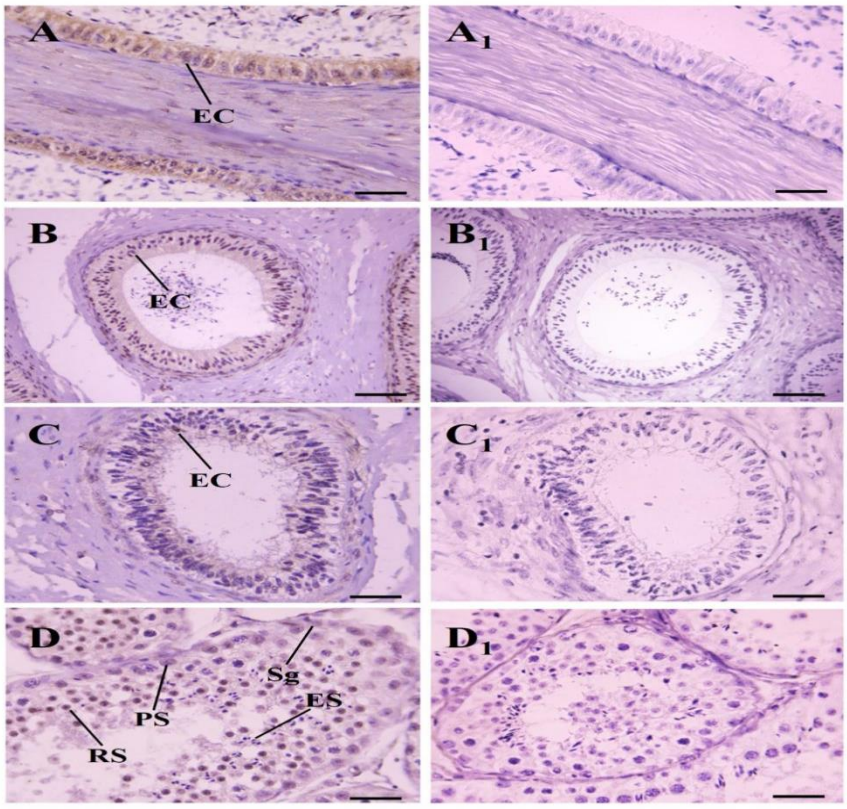


Figure 6: Immunohistochemical staining of pigment epithelium-derived factor in sheep gonadal tissues. The images in the left side with no subscript indicates the experimental group; and those in the right side with subscript 1 indicates the respective negative control. A, caput epididymides; B, corpora epididymides; C, cauda epididymides; D, testes. EC, epithelial cell; Sg, spermatogonia; PS, primary spermatocyte; ES, elongated spermatids; RS, round spermatids. Bar = 25 μm.

4. Discussion

In the present study, detection with RT-PCR, western blotting, and immunohistochemistry confirmed the widespread expression of PEDF in sheep tissues at both mRNA and protein levels. Immunohistochemistry results showed that the PEDF was distributed in all sheep visceral tissues, including the connective tissue between cytoplasm and myocardial cells in the visceral tissues, nerve fibers in brain tissue, and the cytoplasm in testicular and epididymal epithelial cells. The PEDF has been detected in the human liver, testes, stomach, ovaries, prostate, eyes, heart, colon, brain, and spinal cord (Filleur et al., 2009; Xi, 2020). In the current study with sheep as a representative species, we found that PEDF was widely distributed in mammalian visceral tissues, and that the expression of PEDF varied among different visceral tissues. The PEDF exhibits cardioprotective effects against ischemic injury by reducing vascular permeability, apoptosis of cardiomyocytes, and myocardial infarct size (Zhang et al., 2015). It has been confirmed that PEDF is also expressed in liver, serum, subcutaneous adipose tissue and visceral adipose tissue of dairy cows, with no time-related differences in the mRNA abundance of PEDF in the liver of dairy cows (Sadri et al., 2018). As a potent anti-angiogenic agent, PEDF plays a key role in the development and maintenance of the hepatic vascular architecture. In this respect, PEDF has significant therapeutic implications for hepatocellular carcinoma, a typically highly vascularized tumor (Akiba et al., 2021).

In diabetic nephropathy, (Wang et al., 2008) confirmed that PEDF can protect the kidney structure and function from diabetic damage through its anti-inflammatory activity, indicating that PEDF is an endogenous anti-inflammatory factor in the kidney, providing nephroprotective effects in diabetic nephropathy (Li et al., 2019). Results in this study that the PEDF was expressed in sheep visceral tissues suggest that sheep as the model animal is representative to a certain extent. The PEDF was expressed at varying degrees in the heart, liver, and kidney of sheep, which was correlated with the level of neovascularization in these respective tissues. Our study confirmed that PEDF expression was high in the spleen, the largest immune organ in the body and a major site of hematopoiesis during embryonic development. The spleen retains a small number of hematopoietic stem cells even during adult life. The expression of PEDF in spleen cells is consistent with the findings of other studies that the PEDF is expressed in the blood and exhibits certain immune functions (Takanohashi et al., 2005). Lung tissue is highly vascularized, and PEDF expression can also be detected in the blood (Zamiri et al., 2006), suggesting that vascularization of lung tissue may be the cause of high PEDF expression in the lungs. Previous studies have confirmed that PEDF is involved in the development and progression of many respiratory diseases, such as lung cancer (S. X. Zhang et al., 2006), pulmonary fibrosis (Shih et al., 2017), and

chronic obstructive pulmonary disease (Miao et al., 2022). Regarding lung cancer, PEDF inhibits cancer cell proliferation and promotes apoptosis in A594 human lung adenocarcinoma cells (Qin et al., 2022). Vascular endothelial growth factor is an important stimulator of vascular proliferation, and PEDF, as the strongest inhibitor of angiogenesis (Li et al., 2015), inhibited the proliferation but promoted the apoptosis of lung cancer cells (L. Zhang et al., 2006). In this study, we observed that PEDF expression was the highest in the lung, and its expression was localized to the cytoplasm of alveolar macrophages. Thus, PEDF may play an important regulatory role in lung neovascularization. In addition to its anti-angiogenic effects, PEDF has a variety of other biological functions. Neuronal degeneration and death are common features of many neurological diseases and study of the expression and distribution of PEDF in the nervous system can help better understand the neurotrophic role of PEDF. Existing studies have shown that the PEDF mRNA is widely distributed in the hypothalamic substantia nigra, subthalamic nucleus, and medulla of the adult brain and spinal cord (Li et al., 2014), and that PEDF is expressed in almost all brain regions (Tombran-Tink et al., 1996).

Similar to the human brain, PEDF is expressed in most regions of the mouse brain, such as the cerebral cortex and cerebellum (Tombran-Tink & Barnstable, 2003). Consistent with these reports, we found that PEDF was distributed in the cerebrum and cerebellum of male sheep, suggesting that PEDF may have an important biological role in the nervous system. Studies have shown that PEDF expression in the cerebral cortex significantly decreases after brain injury caused by transient ischemia. In a rat model of cerebral infarction caused by transient middle cerebral artery occlusion, the transfection of human PEDF attenuated MCAO-induced neuronal, astrocyte, and oligodendrocyte degeneration, demonstrating the endogenous neuroprotective effect of PEDF on nerve cells in cerebral ischemia. In a normal rat cerebellum, the PEDF mRNA and protein co-localize with calcium-binding proteins in the Purkinje cell layer, confirming that the PEDF mRNA and protein are expressed by Purkinje cell neurons in the adult rat cerebellum. In a model of kainic acid-induced cerebellar injury, the number of calbindin-positive Purkinje neurons was severely lower and the number of glial fibrillary acidic protein-positive astrocytes, which is the most abundant glial cell type in the mammalian brain, increased. These neurons play a key role in central nervous system development, inflammation, and repair (Sanagi et al., 2007) through the production of several cytokines, chemokines, and growth factors (Sanagi et al., 2008). The PEDF expression levels remained significantly low after two days of treatment, and the PEDF mRNA and protein levels significantly increased after seven days of treatment (Zhao & Schwartz, 1998), indicating that an increased PEDF expression in cerebellar injury may constitute one of the compensatory mechanisms against neuronal degeneration. The role of PEDF in tissues and organs rich in blood vessels is mostly related to inhibition of angiogenesis.

Study of the expression and distribution of PEDF in the brain tissues can help further demonstrate the endogenous protective effect and neurotrophic effect of PEDF on nerve cells. Similarly, the fertility of male animals depends on spermatogenesis, which occurs in the seminiferous tubules of the testis, a chamber devoid of blood vessels. Understanding the expression and distribution of PEDF in the gonadal tissues can provide insights into the other biological functions of PEDF, aside from its anti-angiogenic effects. The results of this study showed that PEDF was expressed in the cytoplasm of round spermatocytes of testis, but weakly expressed in spermatocytes, primary spermatocytes and spermatogonocytes. It is expressed in the epithelial cell cytoplasm of the head, body and tail of the epididymis, with highest expression of the PEDF expression in the caput epididymides. By culturing the peritubular cells of the human testis tube wall and studying their secretory factors, it was found that the peritubular cells secrete several important factors, including PEDF (Becerra, 1997; Holekamp et al., 2002; Jablonski et al., 2000; TOMBRAN-TINK, 1991) . The PEDF is expressed in human semen, which is composed of secretions derived from the testes and epididymides .. (Barnstable & Tombran-Tink, 2004; Miller, 2019). Studies have shown that PEDF can stimulate the growth of benign prostatic hyperplasia stromal cells in vitro and enter the human vascular secretory system (Grippo et al., 2012). In studies on the human testes, PEDF was found to be present in the testis tissue, particularly in the testicular pericytes and extracellular matrix. These findings suggest that vascular ischemia in the vas deferens may be due to PEDF secretion by perivasal cells (Orgaz et al., 2009). Existing studies have shown that mouse peritubular cells produce PEDF and that PEDF receptors are present in tubular cells, suggesting that PEDF has a paracrine role in testis development. The PEDF is expressed at high levels in immature mouse testis, and its expression levels subsequently decline in adult mice, suggesting that changes in PEDF expression may be regulated by hormones (Grayhack et al., 2004). Combined with the results of this study, the high expression of PEDF in the caput epididymides suggests that PEDF may play an important biological function in sperm processing and maturation. The expression of PEDF in brain tissues may have a regulatory effect on the secretion of related hormones in the gonadal tissues. The biological function of PEDF is strongly related with the location of its expression. This study confirmed that PEDF is widely expressed in tissues of sheep. Expression and localization of PEDF in mammals can help elucidate its biological functions and provide theoretical support for the application of PEDF in the diagnosis and treatment of various diseases, but the molecular mechanism through which PEDF exerts its multiple bioactive functions is largely unknown. Whether there exists a correlation between the expression of PEDF in brain tissue and gonadal tissue is not clear. Future work is warranted to clarify the biological function and mechanism of PEDF in different visceral tissues.

5. Conclusion

This comprehensive study on the expression and distribution of Pigment Epithelium-Derived Factor (PEDF) in various sheep tissues has provided significant insights into the widespread presence and diverse functional roles of this protein. Our findings confirm that PEDF is expressed across a broad spectrum of tissues, including vital organs and regions integral to athletic performance such as the lungs, heart, and various brain and gonadal tissues. The differential expression levels observed highlight the protein's potential involvement in a range of physiological processes, from angiogenesis and neuroprotection to inflammatory response modulation. Particularly noteworthy is the pronounced expression of PEDF in lung tissues and the cerebrum, suggesting a critical role in respiratory function and neural health, which are of paramount importance in athletic performance and recovery.

The variations in expression between different tissues further emphasize the need for targeted studies to explore the specific roles of PEDF in these areas, especially considering the potential for PEDF to enhance tissue repair and regeneration, key aspects of sports medicine. Moreover, the consistent trends observed at both the mRNA and protein levels reinforce the reliability of PEDF as a biomarker and therapeutic target in veterinary and human medicine alike. The study also underscores the importance of precise surgical and post-operative management in utilizing PEDF for therapeutic purposes, especially in sports-related applications where enhanced recovery and minimal downtime are crucial. Future research should focus on the functional assays of PEDF in in vivo models under various physiological and pathological states to elucidate its exact mechanisms of action.

Additionally, exploring the therapeutic manipulation of PEDF, possibly through gene therapy or recombinant protein administration, could open new avenues for treating and managing sports injuries and other musculoskeletal conditions. The insights gained from such studies could be instrumental in harnessing PEDF's full potential in enhancing athlete care and rehabilitation strategies, ultimately contributing to better health outcomes and performance in sports settings.

Funding

This work was supported by the National Natural Science Foundation of China (32273089, 31872532 and 31272628)

Institutional Review Board Statement

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of Shanxi Agricultural University (2017(050)).

REFERENCES

Akiba, J., Yoshida, T., Sadashima, E., Murata, K., Matsui, T., Yamagishi, S.-I., Kusano, H., Mihara, Y., Mizuochi, S., & Kinjou, Y. (2021). The expression of PEDF and its putative receptors in hepatocellular carcinoma and background liver tissue. *Anticancer research*, *41*(3), 1203-1212.

Barnstable, C. J., & Tombran-Tink, J. (2004). Neuroprotective and antiangiogenic actions of PEDF in the eye: molecular targets and therapeutic potential. *Progress in retinal and eye research*, *23*(5), 561-577.

Becerra, S. P. (1997). Structure-function studies on PEDF: a noninhibitory serpin with neurotrophic activity. *Chemistry and biology of serpins*, 223-237.

Cao, W., Tombran-Tink, J., Chen, W., Mrazek, D., Elias, R., & McGinnis, J. (1999). Pigment epithelium-derived factor protects cultured retinal neurons against hydrogen peroxide-induced cell death. *Journal of neuroscience research*, *57*(6), 789-800.

Carrell, R. W., Evans, D. L., & Stein, P. E. (1991). Mobile reactive centre of serpins and the control of thrombosis. *Nature*, *353*(6344), 576-578.

Filleur, S., Nelius, T., De Riese, W., & Kennedy, R. (2009). Characterization of PEDF: a multi-functional serpin family protein. *Journal of Cellular Biochemistry*, *106*(5), 769-775.

Grayhack, J. T., Smith, N. D., Ilio, K., Wambi, C., Kasjanski, R., Crawford, S. E., Doll, J. A., Wang, Z., Lee, C., & Kozlowski, J. M. (2004). Pigment epithelium-derived factor, a human testis epididymis secretory product, promotes human prostate stromal cell growth in culture. *The Journal of urology*, *171*(1), 434-438.

Grippo, P. J., Fitchev, P. S., Bentrem, D. J., Melstrom, L. G., Dangi-Garimella, S., Krantz, S. B., Heiferman, M. J., Chung, C., Adrian, K., & Cornwell, M. L. (2012). Concurrent PEDF deficiency and Kras mutation induce invasive pancreatic cancer and adipose-rich stroma in mice. *Gut*, *61*(10), 1454-1464.

Holekamp, N. M., Bouck, N., & Volpert, O. (2002). Pigment epithelium-derived factor is deficient in the vitreous of patients with choroidal neovascularization due to age-related macular degeneration. *American journal of ophthalmology*, *134*(2), 220-227.

Jablonski, M. M., Tombran-Tink, J., Mrazek, D. A., & Iannaccone, A. (2000). Pigment epithelium-derived factor supports normal development of photoreceptor neurons and opsin expression after retinal pigment epithelium removal. *Journal of Neuroscience*, *20*(19), 7149-7157.

Li, C., Huang, Z., Zhu, L., Yu, X., Gao, T., Feng, J., Hong, H., Yin, H., Zhou, T., & Qi, W. (2019). The contrary intracellular and extracellular functions of PEDF in HCC development. *Cell Death & Disease*, *10*(10), 742.

Li, L., Yao, Y.-C., Fang, S.-H., Ma, C.-Q., Cen, Y., Xu, Z.-M., Dai, Z.-Y., Li, C., Li, S., & Zhang, T. (2014). Pigment epithelial-derived factor (PEDF)-

triggered lung cancer cell apoptosis relies on p53 protein-driven Fas ligand (Fas-L) up-regulation and Fas protein cell surface translocation. *Journal of Biological Chemistry*, *289*(44), 30785-30799.

Li, X., Wang, T., Yang, T., Shen, Y., An, J., Liu, L., Dong, J., Guo, L., Li, D., & Zhang, X. (2015). Elevated plasma levels of pigment epithelium-derived factor correlated with inflammation and lung function in COPD patients. *International Journal of Chronic Obstructive Pulmonary Disease*, 587-594.

Miao, H., Hui, H., Li, H., Lin, Y., Li, D., Luo, M., Jiang, B., & Zhang, Y. (2022). PEDF inhibits non-small cell lung cancer proliferation by suppressing autophagy through downregulation of AMPK-ULK1 signaling. *Oncology Reports*, *48*(6), 1-11.

Miller, R. B. (2019). Alterity, Intimacy, and the Cultural Turn in Religious Ethics: A Response to Four Critics. *Journal of Religious Ethics*, *47*(1), 203-216.

Orgaz, J., Ladhani, O., Hoek, K., Fernandez-Barral, A., Mihic, D., Aguilera, O., Seftor, E., Bernad, A., Rodriguez-Peralto, J., & Hendrix, M. (2009). Loss of pigment epithelium-derived factor enables migration, invasion and metastatic spread of human melanoma. *Oncogene*, *28*(47), 4147-4161.

Pak, S. C., Kumar, V., Tsu, C., Luke, C. J., Askew, Y. S., Askew, D. J., Mills, D. R., Brömme, D., & Silverman, G. A. (2004). SRP-2 is a cross-class inhibitor that participates in postembryonic development of the nematode Caenorhabditis elegans: initial characterization of the clade L serpins. *Journal of Biological Chemistry*, *279*(15), 15448-15459.

Perez-Mediavilla, L. A., Chew, C., Campochiaro, P. A., Nickells, R. W., Notario, V., Zack, D. J., & Becerra, S. P. (1998). Sequence and expression analysis of bovine pigment epithelium-derived factor. *Biochimica et Biophysica Acta (BBA)-Gene Structure and Expression*, *1398*(2), 203-214.

Qin, X., Jia, C., Liang, J., Chen, J., Liu, X., Chao, Z., Qin, H., Yuan, Y., Liu, Z., & Zhang, Z. (2022). PEDF is an antifibrosis factor that inhibits the activation of fibroblasts in a bleomycin-induced pulmonary fibrosis rat model. *Respiratory Research*, *23*(1), 100.

Sadri, H., Saremi, B., Dänicke, S., Rehage, J., Mielenz, M., Hosseini, A., & Sauerwein, H. (2018). Lactation-related changes in tissue expression of PEDF in dairy cows. *Domestic animal endocrinology*, *64*, 93-101.

Sanagi, T., Yabe, T., & Yamada, H. (2007). Changes in pigment epithelium-derived factor expression following kainic acid induced cerebellar lesion in rat. *Neuroscience letters*, *424*(1), 66-71.

Sanagi, T., Yabe, T., & Yamada, H. (2008). Gene transfer of PEDF attenuates ischemic brain damage in the rat middle cerebral artery occlusion model. *Journal of neurochemistry*, *106*(4), 1841-1854.

Shih, S.-C., Ho, T.-C., Chen, S.-L., & Tsao, Y.-P. (2017). Pigment epithelium-derived factor (PEDF) peptide promotes the expansion of hepatic stem/progenitor cells via ERK and STAT3-dependent signaling.

American Journal of Translational Research, *9*(3), 1114.

Takanohashi, A., Yabe, T., & Schwartz, J. P. (2005). Pigment epithelium-derived factor induces the production of chemokines by rat microglia. *Glia*, *51*(4), 266-278.

TOMBRAN-TINK, J. (1991). PEDF: a pigment epithelium-derived factor with potent neuronal differentiative activity. *Exp Eye Res*, *53*, 411-414.

Tombran-Tink, J., & Barnstable, C. J. (2003). PEDF: a multifaceted neurotrophic factor. *Nature Reviews Neuroscience*, *4*(8), 628-636.

Tombran-Tink, J., & Johnson, L. V. (1989). Neuronal differentiation of retinoblastoma cells induced by medium conditioned by human RPE cells. *Investigative ophthalmology & visual science*, *30*(8), 1700-1707.

Tombran-Tink, J., Mazuruk, K., Rodriguez, I. R., Chung, D., Linker, T., Englander, E., & Chader, G. J. (1996). Organization, evolutionary conservation, expression and unusual Alu density of the human gene for pigment epithelium-derived factor, a unique neurotrophic serpin. *Mol Vis*, *2*(11), 11.

Tombran-Tink, J., Pawar, H., Swaroop, A., Rodriguez, I., & Chader, G. J. (1994). Localization of the gene for pigment epithelium-derived factor (PEDF) to chromosome 17p13. 1 and expression in cultured human retinoblastoma cells. *Genomics*, *19*(2), 266-272.

Travis, J., & Salvesen, G. (1983). Human plasma proteinase inhibitors. *Annual review of biochemistry*, *52*(1), 655-709.

Wang, J. J., Zhang, S. X., Mott, R., Chen, Y., Knapp, R. R., Cao, W., & Ma, J.-x. (2008). Anti-inflammatory effects of pigment epithelium-derived factor in diabetic nephropathy. *American Journal of Physiology-Renal Physiology*, *294*(5), F1166-F1173.

Wu, Y.-Q., & Becerra, S. P. (1996). Proteolytic activity directed toward pigment epithelium-derived factor in vitreous of bovine eyes. Implications of proteolytic processing. *Investigative ophthalmology & visual science*, *37*(10), 1984-1993.

Wu, Y.-Q., Notario, V., Chader, G. J., & Becerra, S. P. (1995). Identification of pigment epithelium-derived factor in the interphotoreceptor matrix of bovine eyes. *Protein expression and purification*, *6*(4), 447-456.

Xi, L. (2020). Pigment epithelium-derived factor as a possible treatment agent for choroidal neovascularization. *Oxidative Medicine and Cellular Longevity*, *2020*.

Zamiri, P., Masli, S., Streilein, J. W., & Taylor, A. W. (2006). Pigment epithelial growth factor suppresses inflammation by modulating macrophage activation. *Investigative ophthalmology & visual science*, *47*(9), 3912-3918.

Zhang, H., Wang, Z., Feng, S.-J., Xu, L., Shi, H.-X., Chen, L.-L., Yuan, G.-D., Yan, W., Zhuang, W., & Zhang, Y.-Q. (2015). PEDF improves cardiac function in rats with acute myocardial infarction via inhibiting vascular permeability and cardiomyocyte apoptosis. *International Journal of*

Molecular Sciences, *16*(3), 5618-5634.

Zhang, L., Chen, J., Ke, Y., Mansel, R. E., & Jiang, W. G. (2006). Expression of pigment epithelial derived factor is reduced in non-small cell lung cancer and is linked to clinical outcome. *International journal of molecular medicine*, *17*(5), 937-944.

Zhang, S. X., Wang, J. J., Gao, G., Shao, C., Mott, R., & Ma, J. x. (2006). Pigment epithelium-derived factor (PEDF) is an endogenous antiinflammatory factor. *The FASEB journal*, *20*(2), 323-325.

Zhao, B., & Schwartz, J. P. (1998). Involvement of cytokines in normal CNS development and neurological diseases: recent progress and perspectives. *Journal of neuroscience research*, *52*(1), 7-16.

Zhou, A., Wei, Z., Read, R. J., & Carrell, R. W. (2006). Structural mechanism for the carriage and release of thyroxine in the blood. *Proceedings of the National Academy of Sciences*, *103*(36), 13321-13326.