Molecular structure, gene expression and functional role of WFDC1 in angiogenesis and cancer

Running title: The role of WFDC1 in angiogenesis and cancer

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Abstract

Whey acidic proteins (WAP) perform a diverse range of important biological functions, including proteinase activity, calcium transport and bacterial growth. The WAP four-disulfide core domain protein 1 (WFDC1) gene (also called PS20), encodes the 20 kDa prostate stromal protein (ps20), which is a member of the WAP-type four disulfide core domain family of proteins, and exhibits characteristics of serine protease inhibitors, such as elafin and secretory leucocyte protease inhibitor (SLPI). Molecular structural analysis reveals that ps20 consists of four disulfide bonds formed by eight cysteine residues located at the carboxyl terminus of the protein. Wfdc1-null mice were found to display no overt developmental phenotype, suggesting a dispensable role in organ growth and development. However, WFDC1 was able to mediate endothelial cell migration and pericyte stabilization, which are vital for the formation of functional vascular structures. WFDC1 was also found to be downregulated in cancers and exhibited a regulatory effect on cell proliferation. In addition, it was involved in the modulation of memory T cells during human immunodeficiency virus (HIV) infection. Gaining a solid understanding of the mechanisms by which WFDC1 regulates tissue homeostasis and disease processes, in a tissue specific manner, will be an important move towards the development of WFDC1/ps20 as potential therapeutic targets.

Key words: mesenchymal cell, foetal, healing, inflammation, regeneration, metastasis

Introduction

Whey acidic proteins (WAP) are widely conserved among various species, and play important roles in diverse biological processes, such as proteinase activity, calcium transport and bacterial growth.[1] The WAP four-disulfide core domain protein 1 (WFDC1) gene encodes an approximately 20 kDa protein, called prostate stromal protein 20 (ps20).[2] ps20 was first identified as a secreted factor from foetal urogenital sinus mesenchymal cells, and exhibited growth inhibitory activity towards bladder and prostate epithelial cells in vitro.[3] WFDC1 is located cytogenetically at chromosome 16q24.1, a locus in which frequent loss of heterozygosity was detected in many cancer types.[4] WFDC1 appears to be downregulated in cancers, and might play a protective role by inhibiting cancer cell proliferation.[5-7] Intriguingly, WFDC1 was able to mediate endothelial cell migration and pericyte stabilization, which are significant for the formation of vascular structures in prostate cancer.[8] It is also involved in the modulation of memory T cells during human immunodeficiency virus (HIV) infection. Surprisingly, Wfdc1-null mice exhibited a lack of developmental phenotypes,[9] suggesting it is dispensable during the developmental stages, however, appears to be involved with the regulation of inflammation and repair processes.[9] The aim of this review is to provide a current overview regarding the role of WFDC1, including molecular structure, gene expression and function. It is revealed that WFDC1 is involved in the regulation of angiogenesis and cancers, as well as immunity and infection. Advancing our understanding of the molecular mechanisms by which WFDC1 regulates tissue homeostasis and disease in a tissue specific manner will be important for the development of WFDC1 as a therapeutic target.

The molecular structure and expression of WFDC1

WFDC1 is also known as PS20,[10-12] and its precursor protein is processed by cathepsin L and transglutaminase to form the mature and functional protein.[7] Multiple sequence alignment shows that WFDC1 shares a high degree of amino acid sequence identity among species including human, Pan troglodytes (Chimpanzee), rat, mouse, chicken, sheep and Danio rerio (Zebrafish), which is indicative of its highly conserved nature (Figure 1A, B). In addition, human WDFC1 shares only very limited sequence homology with other serine protease inhibitors, such as elafin and secretory leucocyte protease inhibitor (SLPI), suggesting it may have a unique functional role (Figure 2A, B).

Molecular structure analyses reveal that human WFDC1 contains a signal sequence (amino acid residues 1-31), a WAP domain (amino acid residues 59 – 108), and two poly proline motifs (amino acid residues 67 – 70; 109 – 112) (Figure 3A). The WAP domain shares the predicted secondary structure of D1udka, based on the dictionary of secondary structure of proteins (SDDP) by the Phyre2 web portal for protein modelling[13] (Figure 3B, C). It consists of four disulfide bonds folded by eight cysteine residues present at the carboxyl region of the protein. Bioinformatics analysis reveals that these four-disulfide bonds are present in amino acid residues, 66 ↔ 96, 78 ↔ 100, 83 ↔ 95, 89 ↔ 104, and are predicted to be involved in the binding of proteinases with other molecules. Further, recent research indicates the regions corresponding to exons 3 and 4 are ps20-specific structure-function modules, with C96, R94, L105, and C66 vital for the integrity of this ps20 region.[14] Investigation of the post-translational regulatory apparatus of WFDC1 found that ps20 was cleaved by cathepsin L (CL) at the carboxyl terminus, which might affect its function in wound healing by a reduced capacity for multimerization and cross-linking to fibronectin and

glycosaminoglycans of the extracellular matrix. However, the growth inhibitory function of WFDC1/ps20 appears to be retained following cleavage by CL.[15]

Northern blot tissue expression analysis found that WFDC1 was most highly expressed in the heart, whilst immunohistochemical localization of ps20 showed a cell-specific expression pattern in visceral and vascular smooth muscle in all tissues, including the prostate gland.[16] WFDC1 transcripts were also found to be expressed in the lens, retina, and optic nerves of embryonic and adult mouse eyes, which indicates of a role of WFDC1 in mammalian eye development, and possibly in disorders of the eye.[17] WFDC1 expression appears to be negatively regulated by estrogens and tamoxifen,[18] but positively induced by TGF-β1[8] and by glycogen synthase kinase 3β (GSK-3β) inhibitor in ciliary epithelium.[19] It is postulated that WFDC1 is able to upregulate Dickkopf-1 (Dkk1) gene expression,[20] which may inhibit Wnt signalling and affect the suppression of tumour growth. Additionally, ps20 may be a signature marker of HIV susceptible CD4 T cells, involving enhanced CD54 integrin expression,[12] which indicates a potential anti-HIV therapeutic strategy aiming to disrupt ps20 function and downstream signalling. Further research is needed to determine the molecular mechanisms of WFDC1/ps20 signalling pathways.

Through conducting Genevisible®- based bioinformatics analyses, WFDC1 mRNA expression was detected in human and mouse tissues and cells.[21] In human tissues, WFDC1 mRNA was most abundantly expressed in umbilical cord, foetal retina pigment epithelium, and foetal retina pigment epithelium cells (Figure 4A). In mouse tissues, it was most highly expressed in brain microvessel, cerebellar microvessel and blood vessel (Figure 4B), which is consistent with its emerging role in angiogenesis.[2,8]

The role of WFDC1 in angiogenesis

As a secreted protein from prostate stromal cells, WFDC1/ps20 was found to promote vascular density in a differential reactive stroma (DRS) prostate cancer xenograft model.[2,8] In vitro findings suggested that WFDC1 might stimulate the migration of endothelial cells, but has no significant effect on the proliferation of endothelial cells.[8] WFDC1/ps20-treated tumours showed an increase in microvessel density, which was accompanied by an augmentation in weight and volume.[8] WFDC1 mRNA expression was shown to be induced by TGF-β1, a recognized regulator of stromal induced angiogenesis and endothelial cell-pericyte interactions in DRS tumours, which together suggests that WFDC1/TGF-β1 signalling might be involved in the regulation of angiogenesis.[8]

In a study of disease susceptibility of human age-related macular degeneration (ADM), the WFDC1 gene was found to be differentially expressed in the retinal pigmented epithelium/choroid (RPEC).[22] Increased expression of WFDC1 in RPEC tissues may indicate a role of WFDC1 in the pathogenesis of AMD, which is usually accompanied by neovascular overgrowth under the central retina.[22] Consistently, a one-nucleotide insertion mutation of the WFDC1 gene, resulting in a frameshift mutation and a premature termination codon at the middle of the protein, was associated with multiple ocular defects (MOD) in cattle, a disorder characterized by lens dysplasia, retinal detachment, microphthalmia, and impaired hyaloid artery.[17] Recently, WFDC1 was identified as a putative endothelial cell-specific Wnt/β-catenin-responsive extracellular matrix signature gene in mouse forebrain, which might contribute to the blood-brain barrier differentiation during forebrain development.[23]

Taken together, these studies suggest that WFDC1 might be involved in the regulation of angiogenesis and the development of vascular structures. Further research is required to investigate these possibilities, including the receptor, signalling mechanisms and pathways by which WFDC1 might affect these vital biological processes. Such future studies might discover novel therapeutic interventions targeting WFDC1/ps20 signalling directly and downstream effectors.

The role of WFDC1 in prostate cancer and other cancers

WAP family proteins have been linked with multiple types of cancer.[24] In prostate tumours, WFDC1 was found to be downregulated when compared to normal prostate tissue,[11,25] arguing for a putative role as a tumour suppressive gene. Additional research of the altered expression profile of WFDC1 in prostate cancer revealed that decreased stromal WFDC1/ps20 expression coincided with the development of prostate cancer reactive stroma, and that increased epithelial WFDC1/ps20 expression was associated with a more aggressive epithelial phenotype, which is required for the process of epithelial mesenchymal transition (EMT).[10] More recently, in vitro findings determined that WFDC1/ps20 signalling in human prostate stromal cells and human lymph node carcinoma of the prostate (LNCaP) cells is dependent on cyclooxygenase-2 (COX-2), indicating that expression of WFDC1/ps20 in prostate stroma may regulate epithelial and tissue growth, and suppress tumour progression, via the prostaglandin synthase pathway.[7] Further research is needed to investigate these findings, and to develop the novel immunotherapeutic potential of WFDC1 for the treatment of prostate cancer.[26]

WFDC1 expression has been found to be significantly reduced consistently across various forms of cancer, including fibrosarcomas, and tumours of the lung, bladder, and brain.[5] Cancer-associated fibroblasts (CAFs), located within the stroma adjacent to the tumour, are thought to cultivate the cancer transformation process, and WFDC1 was found to be dramatically under-expressed by CAFs and stromal mesenchymal cells.[5] In vitro findings confirmed that CAFs proliferate more rapidly than normal fibroblasts; and overexpression of WFDC1 inhibited the growth of HT1080 fibrosarcoma cells.[5] WFDC1 level was upregulated in senescent fibroblasts.[5] These studies imply a role for WFDC1 in inhibiting the proliferation of both tumours and senescent cells, and as a biomarker for cellular transformation.[5] Further research is needed to determine how WFDC1/ps20, a protease inhibitor, may inhibit cancer progression.

In melanoma tumorigenesis, WFDC1 was uncovered to display a tumour suppressive effect owing at least in part to the up-regulation of Dkk1 gene expression, a potent inhibitor of the Wnt signalling pathway.[20] Promoter hypermethylation was found to be a significant mechanism for silencing of WFDC1 expression in melanoma cell lines.[20] Interestingly, related research utilizing quantitative methylation-specific PCR, found a lower frequency of WFDC1 methylation in melanoma cell lines,[27] indicating the need for further research to investigate the regulatory role of WFDC1 promoter methylation in melanoma progression.

In association with ovarian cancer, an upregulation of WFDC1 expression was found in ovarian cell lines with PAX2 gene knockdown, accompanied by reduced cell proliferation and migration.[28] Post-transcriptional stabilization of WFDC1 was found to be a possible mechanism for the suppression of ovarian cancer invasion and metastasis.[29] Mechanistic studies of RNA-binding protein (RBP), sorbin and SH3 domain containing 2 (SORBS2),

indicate that SORBS2 may bind and stabilize the 3' untranslated region of WFDC1, resulting in decreased ovarian cancer invasiveness of the tumour microenvironment.[29] Taken together, WFDC1 appears to be a post-transcriptional network target for immunomodulation and the inhibition of cancer progression, indicating its therapeutic potential. Further investigation of the post-transcriptional regulation of WFDC1 by RBPs, including SORBS2, is required for additional forms of cancer, such as hepatocellular carcinoma (HCC).[30]

Using Genevisible® -based bioinformatics analysis, WFDC1 mRNA expression was detected in human and mouse cancers.[21] In human tissues, WFDC1 mRNA was most abundantly expressed in dedifferentiated chondrosarcoma, prostate carcinoma, and retroperitoneum leiomyosarcoma (Figure 5A). In mouse tissues, it was expressed by pancreas infiltrating duct carcinoma, neoplasms of digestive organs and hepatocellular carcinoma (Figure 5B). However, the exact expression profile and role of WFDC1 in these diverse types of cancer remains to be fully elucidated.

The role of WFDC1 in inflammation and infection

WFDC1 was found to modulate the inflammatory and wound healing responses.[9] In skin wounding experiments, Wfdc1-null mice exhibited an elevated rate of skin closure, which was associated with elevated deposition of osteopontin and macrophage recruitment.[9] Interestingly, SNP variants near WFDC1 showed significant evidence for epistatic interactions with PTPN22, a well-established genetic factor (PTPN22 1858T) in rheumatoid arthritis (RA), indicating its possible involvement in the susceptibility to RA.[31] Given the nature of inflamed tissues in RA disease, the pathological involvement of WDFC1 in RA may be attributed to its role in the regulation of inflammation. However, the precise

mechanism by which WFDC1 regulates disease susceptibility of RA requires further investigation.

WFDC1 was shown to increase HIV infectivity by potentiating HIV entry via cell fusion and increased CD54 integrin expression.[12,32] Consistently, these effects were neutralised by anti-PS20 antibody.[12] Further, siRNA mediated WFDC1 knockdown or neutralizing antibody to WFDC1 attenuated HIV transfer. In contrast, exogenous addition of recombinant PS20 protein or ectopic forced expression of WFDC1 improves HIV transfer.[33] Consistently, neutrophils isolated from wfdc1/ps20 deficient mice were found to be more susceptible to mouse hepatitis virus strain 1 (MHV-1) infection compared to neutrophils isolated from normal ps20 mice.[34] Collectively, these findings suggest a modulating role of WFDC1 in the pathogenesis of HIV infectivity.

Summary

As a member of the WAP family, the ps20 protein shares characteristics of serine protease inhibitors, such as elafin and SLPI. Interestingly, WFDC1 was revealed to mediate endothelial cell migration and pericyte stabilization, which are imperative to the formation of vascular structures. WFDC1 appears to be downregulated in cancers and to exhibit an inhibitory effect on cell proliferation. It is also involved in the modulation of virus entry during HIV infection. However, several questions remain to be addressed regarding the molecular action of WFDC1 in angiogenesis, cancers, and inflammation. Further research elucidating the molecular roadmap of WFDC1 will facilitate the development of therapeutic strategies for the treatment of WFDC-1 related conditions.

Conflict of Interest

The authors have no conflict of interest to declare.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions

Sipin Zhu and Lin Ye conducted research and drafted the manuscript. Samuel Bennett and Huazi Xu provided evaluation and assistance in the process of drafting and revision of the manuscript. Jiake Xu performed the bioinformatics protein structural analysis. Dengwei He and Jiake Xu supervised the study and revised the manuscript.

References

1. Smith VJ. Phylogeny of whey acidic protein (WAP) four-disulfide core proteins and their role in lower vertebrates and invertebrates. *Biochem Soc Trans.* 2011;39(5):1403-1408.
2. Ressler SJ, Rowley DR. The WFDC1 gene: role in wound response and tissue homoeostasis. *Biochem Soc Trans.* 2011;39(5):1455-1459.
3. Rowley DR, Dang TD, Larsen M, Gerdes MJ, McBride L, Lu B. Purification of a novel protein (ps20) from urogenital sinus mesenchymal cells with growth inhibitory properties in vitro. *J Biol Chem.* 1995;270(37):22058-22065.
4. Larsen M, Ressler SJ, Gerdes MJ, et al. The WFDC1 gene encoding ps20 localizes to 16q24, a region of LOH in multiple cancers. *Mamm Genome.* 2000;11(9):767-773.
5. Madar S, Brosh R, Buganim Y, et al. Modulated expression of WFDC1 during carcinogenesis and cellular senescence. *Carcinogenesis.* 2009;30(1):20-27.
6. Li Y, Basang Z, Ding H, et al. Latexin expression is downregulated in human gastric carcinomas and exhibits tumor suppressor potential. *BMC Cancer.* 2011;11:121.
7. Hickman OJ, Smith RA, Dasgupta P, et al. Expression of two WFDC1/ps20 isoforms in prostate stromal cells induces paracrine apoptosis through regulation of PTGS2/COX-2. *Br J Cancer.* 2016;114(11):1235-1242.
8. McAlhany SJ, Ressler SJ, Larsen M, et al. Promotion of angiogenesis by ps20 in the differential reactive stroma prostate cancer xenograft model. *Cancer Res.* 2003;63(18):5859-5865.
9. Ressler SJ, Dang TD, Wu SM, et al. WFDC1 is a key modulator of inflammatory and wound repair responses. *Am J Pathol.* 2014;184(11):2951-2964.

10. McAlhany SJ, Ayala GE, Frolov A, et al. Decreased stromal expression and increased epithelial expression of WFDC1/ps20 in prostate cancer is associated with reduced recurrence-free survival. *Prostate.* 2004;61(2):182-191.
11. Watson JE, Kamkar S, James K, et al. Molecular analysis of WFDC1/ps20 gene in prostate cancer. *Prostate.* 2004;61(2):192-199.
12. Alvarez R, Reading J, King DF, et al. WFDC1/ps20 is a novel innate immunomodulatory signature protein of human immunodeficiency virus (HIV)-permissive CD4+ CD45RO+ memory T cells that promotes infection by upregulating CD54 integrin expression and is elevated in HIV type 1 infection. *J Virol.* 2008;82(1):471-486.
13. Kelley LA, Mezulis S, Yates CM, Wass MN, Sternberg MJ. The Phyre2 web portal for protein modeling, prediction and analysis. *Nat Protoc.* 2015;10(6):845-858.
14. Solís-Calero C, Carvalho HF. Phylogenetic, molecular evolution and structural analyses of the WFDC1/prostate stromal protein 20 (ps20). *Gene.* 2019;686:125-140.
15. Hickman OJ, Dasgupta P, Galustian C, Smith RA, Vyakarnam A. Cathepsin-L and transglutaminase dependent processing of ps20: A novel mechanism for ps20 regulation via ECM cross-linking. *Biochem Biophys Rep.* 2016;7:328-337.
16. Larsen M, Ressler SJ, Lu B, et al. Molecular cloning and expression of ps20 growth inhibitor. A novel WAP-type "four-disulfide core" domain protein expressed in smooth muscle. *J Biol Chem.* 1998;273(8):4574-4584.
17. Abbasi AR, Khalaj M, Tsuji T, et al. A mutation of the WFDC1 gene is responsible for multiple ocular defects in cattle. *Genomics.* 2009;94(1):55-62.
18. Hung H. Suppression of ps20 expression in the rat uterus by tamoxifen and estrogens. *Endocrinology.* 2005;146(5):2388-2396.
19. Kinoshita H, Suzuma K, Kaneko J, Mandai M, Kitaoka T, Takahashi M. Induction of Functional 3D Ciliary Epithelium-Like Structure From Mouse Induced Pluripotent Stem Cells. *Invest Ophthalmol Vis Sci.* 2016;57(1):153-161.
20. Liu S, Howell P, Ren S, et al. Expression and functional analysis of the WAP four disulfide core domain 1 gene in human melanoma. *Clin Exp Metastasis.* 2009;26(7):739-749.
21. Hruz T, Laule O, Szabo G, et al. Genevestigator v3: a reference expression database for the meta-analysis of transcriptomes. *Advances in bioinformatics.* 2008;2008:420747.
22. Radeke MJ, Peterson KE, Johnson LV, Anderson DH. Disease susceptibility of the human macula: differential gene transcription in the retinal pigmented epithelium/choroid. *Exp Eye Res.* 2007;85(3):366-380.
23. Jensen LD, Hot B, Ramskold D, et al. Disruption of the Extracellular Matrix Progressively Impairs Central Nervous System Vascular Maturation Downstream of beta-Catenin Signaling. *Arterioscler Thromb Vasc Biol.* 2019;39(7):1432-1447.
24. Bouchard D, Morisset D, Bourbonnais Y, Tremblay GM. Proteins with whey-acidic-protein motifs and cancer. *Lancet Oncol.* 2006;7(2):167-174.
25. Watson JE, Doggett NA, Albertson DG, et al. Integration of high-resolution array comparative genomic hybridization analysis of chromosome 16q with expression array data refines common regions of loss at 16q23-qter and identifies underlying candidate tumor suppressor genes in prostate cancer. *Oncogene.* 2004;23(19):3487-3494.
26. Galustian C, Vyakarnam A, Elhage O, Hickman O, Dasgupta P, Smith RA. Immunotherapy of prostate cancer: identification of new treatments and targets for therapy, and role of WAP domain-containing proteins. *Biochem Soc Trans.* 2011;39(5):1433-1436.
27. Liu S, Ren S, Howell P, Fodstad O, Riker AI. Identification of novel epigenetically modified genes in human melanoma via promoter methylation gene profiling. *Pigment Cell Melanoma Res.* 2008;21(5):545-558.
28. Song H, Kwan SY, Izaguirre DI, et al. PAX2 Expression in Ovarian Cancer. *Int J Mol Sci.* 2013;14(3):6090-6105.

29. Zhao L, Wang W, Huang S, et al. The RNA binding protein SORBS2 suppresses metastatic colonization of ovarian cancer by stabilizing tumor-suppressive immunomodulatory transcripts. *Genome Biol.* 2018;19(1):35.
30. Han L, Huang C, Zhang S. The RNA-binding protein SORBS2 suppresses hepatocellular carcinoma tumourigenesis and metastasis by stabilizing RORA mRNA. *Liver Int.* 2019;39(11):2190-2203.
31. Briggs FB, Ramsay PP, Madden E, et al. Supervised machine learning and logistic regression identifies novel epistatic risk factors with PTPN22 for rheumatoid arthritis. *Genes Immun.* 2010;11(3):199-208.
32. Drannik AG, Henrick BM, Rosenthal KL. War and peace between WAP and HIV: role of SLPI, trappin-2, elafin and ps20 in susceptibility to HIV infection. *Biochem Soc Trans.* 2011;39(5):1427-1432.
33. Alvarez RA, Thorborn G, Reading JL, Reddy SK, Vyakarnam A. WFDC1 expression identifies memory CD4 T-lymphocytes rendered vulnerable to cell-cell HIV-1 transfer by promoting intercellular adhesive junctions. *Retrovirology.* 2011;8:29.
34. Rogers E, Wang BX, Cui Z, et al. WFDC1/ps20: a host factor that influences the neutrophil response to murine hepatitis virus (MHV) 1 infection. *Antiviral Res.* 2012;96(2):158-168.

Figure Legends

Figure 1. Multiple sequence alignment results showing amino acid sequence identity and similarity among WFDC1 in various species of human, mouse, rat, bovine, and rabbit (A). A family tree of WFDC1 proteins is elucidated (B)

Figure 2. Multiple sequence alignment results showing amino acid sequence similarity among WFDC1, elafin and SLPI (A). A family tree of WFDC1, elafin and SLPI is presented (B)

Figure 3. Molecular structure of WFDC1/ps20 protein. Secondary structure of WFDC1 showing a characteristic of WAP domain and two poly proline regions (A). WFDC1 shows similarity to Elafin-like domain with characteristic folding of knottins such as small inhibitors, toxins, lectins. Forty-nine residues (22% of WFDC1) have been modelled with 99.0% confidence by the single highest scoring with dul1 (B). Tertiary structure analysis

showing a typical WAP family protein predicted by the Phyre2 web portal (http://www.sbg.bio.ic.ac.uk/phyre2/) (C)

Figure 4. mRNA expression profiling of WFDC1 gene in both human (A) and mouse (B) tissues, with ten most highly ranking tissues, based mRNA express levels, predicted by Genevisible® bioinformatics tool (http://genevisible.com)

Figure 5. mRNA expression profiling of WFDC1 gene in both human (A) and mouse (B) cancer samples, with ten most highly ranking cancer tissues, based mRNA expression levels, predicted by Genevisible® bioinformatics tool (http://genevisible.com)