University Library

A gateway to Melbourne's research publications

Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:
George, L;Winship, A;Sorby, K;Dimitriadis, E;Menkhorst, E

Title:
Profilin-1 is dysregulated in endometroid (type I) endometrial cancer promoting cell proliferation and inhibiting pro-inflammatory cytokine production

Date:
2020-10-22

Citation:
George, L., Winship, A., Sorby, K., Dimitriadis, E. & Menkhorst, E. (2020). Profilin-1 is dysregulated in endometroid (type I) endometrial cancer promoting cell proliferation and inhibiting pro-inflammatory cytokine production. Biochemical and Biophysical Research Communications, 531 (4), pp.459-464. https://doi.org/10.1016/j.bbrc.2020.07.123.

Persistent Link:
https://hdl.handle.net/11343/268163

Biochemical and Biophysical Research Communications

Profilin-1 is dysregulated in endometroid (type I) endometrial cancer promoting cell proliferation and inhibiting pro-inflammatory cytokine production

--Manuscript Draft--

Manuscript Number:	BBRC-20-6323
Article Type:	Short communication/Research article
Keywords:	Profilin-1; AN3CA; THP1; Type 1 endometrial cancer; pro-inflammatory cytokine
Corresponding Author:	Ellen Menkhorst University of Melbourne Melbourne, VIC AUSTRALIA
First Author:	Lisanne George
Order of Authors:	Lisanne George
	Amy Winship
	Kelli Sorby
	Evdokia Dimitriadis
	Ellen Menkhorst

Powered by Editorial Manager® and ProduXion Manager® from Aries Systems Corporation

The Editor, *Biochemical and Biophysical Research Communications*,

We respectfully submit our manuscript entitled **‘Profilin 1 is dysregulated in endometroid (type I) endometrial cancer promoting cell proliferation and inhibiting pro-inflammatory cytokine production**’ by George, Winship, Sorby, Dimitriadis and Menkhorst for consideration for publication in Biochemical and Biophysical Research Communications.

Endometrial cancer is the most common female gynaecological cancer worldwide - alarmingly, the incidence and mortality of endometrial cancer is increasing, particularly in younger women of reproductive age. Unfortunately, there are limited treatment options for EC, particularly for recurrent or metastatic disease. Profilin 1 regulates tumorogenesis in numerous cancers but the role of profilin 1 has not been investigated in endometrial cancer.

Profilin 1 immunostaining was significantly reduced in the endometrial epithelial cancer cell compartment of grade II and III endometrial cancer compared to grade I endometrial cancer and normally cycling endometrium. Silencing profilin 1 *in vitro* increased endometrial epithelial cancer cell line (AN3CA) adhesion and proliferation. Profilin 1 immunostaining was strongly observed in infiltrating immune cells of the stromal compartment in endometrial cancer. Profilin 1 inhibited THP1 macrophage pro-inflammatory cytokine expression *in vitro*. Profilin 1 may play a role in the tumorogenesis of endometrial cancer due to increased endometrial epithelial cancer cell proliferation coupled with a pro-tolerance tumor microenvironment.

This an original study presenting novel, unpublished work. The material submitted in this manuscript has not been previously reported and is not under consideration for publication elsewhere. We will not submit this manuscript to another journal until a decision has been reached by Biochemical and Biophysical Research Communications as to its suitability for publication. All the authors concur with the submission. The authors have no conflicting financial or other interests.

Kind Regards,

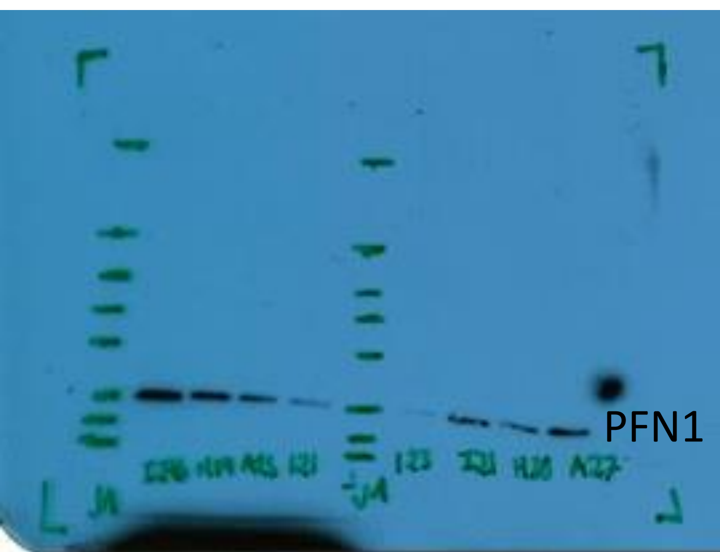
Ellen Menkhorst

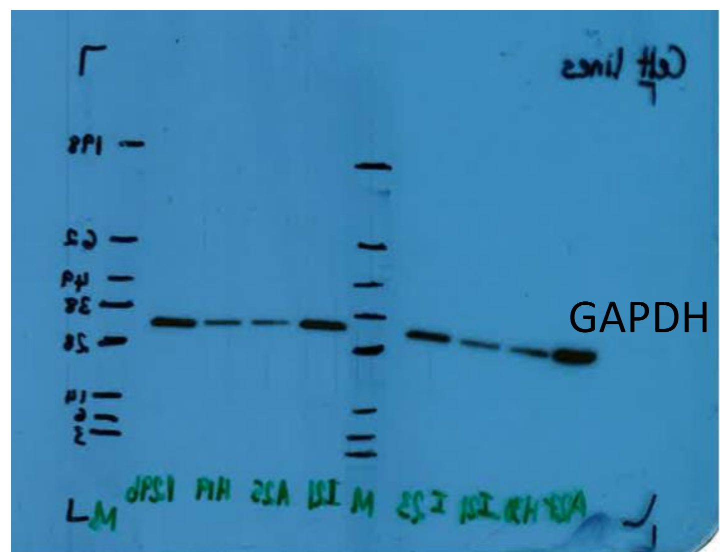
PFN1 GAPDH Western Blot Figure 2B

PFN1 Western blot

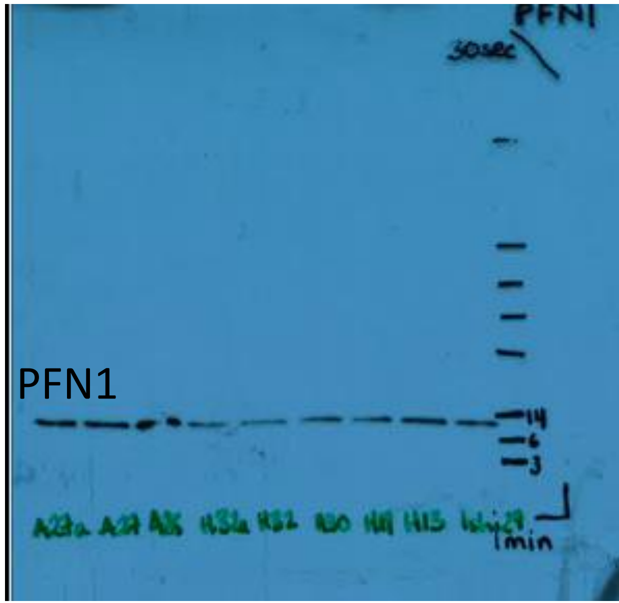
Blot #1

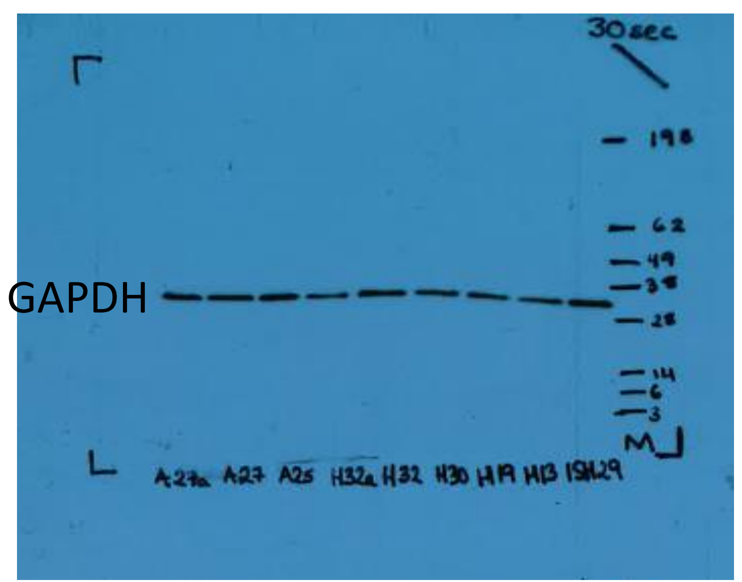
PFN1 and GAPDH for Figure 2B





Blot #2





Highlights

- PFN1 production is dysregulated in Type 1 EC.
- PFN1 protein is lost in Type 1 grade II and III endometrial epithelial cancer cells.
- Silencing PFN1 promoted AN3CA adhesion and proliferation.
- PFN1 is strongly expressed in the stromal compartment of EC.
- PFN1 down-regulated *TNFα* and *IL1β* mRNA expression in THP1 cells

Manuscript

Click here to view linked References

Profilin-1 is dysregulated in endometroid (type I) endometrial cancer promoting cell proliferation and inhibiting pro-inflammatory cytokine production.

Lisanne George[a], Amy Winship[a,b,1], Kelli Sorby[a,c,d,2], Evdokia Dimitriadis[a,b,c,d,2,] and Ellen Menkhorst[a,c,d,2]

[a]Centre for Reproductive Health, Hudson Institute of Medical Research, Clayton, Victoria 3186

[b]Department of Anatomy and Developmental Biology, Monash University, Clayton, Victoria 3800

[c]Department of Obstetrics and Gynaecology, The University of Melbourne, Parkville, VIC, Australia

[d]Gynaecology Research Centre, Royal Women's Hospital, Parkville, VIC, Australia

Corresponding author: Ellen Menkhorst. Level 7, Department of Obstetrics and Gynecology, The Royal Women's Hospital, 20 Flemington Road, Parkville, VIC, Australia, 3052. Email: ellen.menkhorst@unimelb.edu.au; phone number +61383453780;

[1]Present address: Department of Anatomy and Developmental Biology, Monash University, Clayton, Victoria 3800

[2]Present address: Department of Obstetrics and Gynaecology, The University of Melbourne, Parkville, VIC, Australia and Gynaecology Research Centre, Royal Women's Hospital, Parkville, VIC, Australia

Abstract

Endometrial cancer (EC) is the most common gynaecological malignancy. Alarmingly its incidence and mortality rate is increasing particularly in younger women of reproductive age. Despite this, there are limited treatment options for EC. Profilin-1 (PFN1) regulates tumorigenesis in numerous cancers, but the role of PFN1 in EC has not been investigated. We hypothesized that PFN1 would have altered expression in EC and contribute to the development of EC.

We quantified PFN1 in type 1 EC and benign/normal endometrium by RT-qPCR and IHC. The effect of silencing PFN1 on cell adhesion and proliferation was investigated using 2 EC cell lines (HEC1A and AN3CA). The effect of recombinant PFN1 (100μM) on pro-inflammatory cytokine gene expression was investigated using THP1 monocyte cell line.

PFN1 immunolocalized to glandular epithelial cells, vascular endothelial cells and leukocytes in the stromal compartment of normal endometrium and EC. PFN1 immunostaining intensity was significantly elevated in grade (G)I EC compared to normal endometrium, GI-II and GIII EC. In endometrial epithelial cancer cells alone, PFN1 immunostaining intensity was significantly reduced in GII and III EC compared to normal endometrium and GI EC. The stromal compartment of EC had strong PFN1 expression compared to benign and normal endometrium. Silencing PFN1 in the AN3CA endometrial epithelial cancer cell line significantly enhanced cell adhesion and proliferation. PFN1 treatment significantly down-regulated *TNFα* and *IL1β* mRNA expression by THP1 cells.

This study demonstrated that whilst PFN1 production is retained in the stromal compartment of EC, PFN1 production is lost in endometrial epithelial cancer cells with increasing cancer grade. PFN1 may play a role in the tumorigenesis of EC. Loss of PFN1 in GII and GIII endometrial epithelial cancer cells associated with sustained PFN1 by infiltrating immune cells may promote EC tumorigenesis due to increased endometrial epithelial cancer cell proliferation coupled with a pro-tolerance tumor microenvironment.

45 **Keywords**

46 Profilin-1; AN3CA; THP1; Type 1 endometrial cancer;

47 **Abbreviations**

48 PFN1, profilin-1

49 EC, endometrial cancer

50 **Introduction**

51 Endometrial cancer (EC) is the most common female gynaecological cancer worldwide [1]. Of significant
52 concern is the increasing incidence and mortality of EC, especially in reproductive age women [2], likely
53 associated with increased life expectancy and obesity found in developed countries [3]. Therapeutic
54 options beyond hysterectomy are limited for EC, and there are few treatments available for recurrent or
55 metastatic disease [4].

56 The International Federation of Gynecology and Obstetrics guidelines are used to categorize EC. EC can
57 be categorized into type 1 or type 2 based on histology. Over 80% of all EC is type 1 [5], a histologically
58 endometrioid cancer associated with unopposed oestrogen, resulting in endometrial hyperplasia, which
59 is characterized by excess proliferation of endometrial glands causing an increase in the glandular to
60 stroma ratio [6]. Type 1 EC Tumor grade (GI-III) is defined by histology, metastatic behaviour and the
61 degree to which the EC has invaded the uterine corpus and surrounding peritoneum [6]: i) Grade I (GI),
62 well differentiated; ii) grade II (GII), moderately differentiated; and iii) grade III (GIII), poorly differentiated
63 [6]. Type 1 EC is associated with gene mutations in *K-ras* and phosphatase and tensin homolog (PTEN) [4].
64 Type 2 EC are generally higher-grade, more aggressive adenocarcinomas which are non-endometroid in
65 histology [6]. Type 2 EC are not driven by oestrogen and are associated with gene mutations in tumor
66 protein P53 *(p53)* [4]. However, treatments based on histological classifications can be ineffective, in part
67 due to the highly variable gene mutations found in both EC types [7]. Personalized therapies based on
68 molecular characterization of individual tumors may improve patient outcomes [8].

69 Profilin-1 (PFN1) is a small, 15kDa, multi-ligand protein expressed ubiquitously in mammalian cells [9]
70 including endometrial epithelial cells [10, 11]. PFN1 was initially identified as an actin-binding protein and
71 is now recognized as essential for cell survival due to its role in the regulation of the dynamic actin
72 cytoskeleton. PFN1 is also released extracellularly although it doesn’t have a secretion signal motif. PFN1
73 has been detected in dendritic-derived exosomes [12], conditioned media [11, 12] and serum [13].

74 Disruptions to the actin cytoskeleton is a hallmark of cancer cells [14] and PFN1 is dysregulated in multiple
75 cancers [9], however PFN1 has cancer dependent functions in regulating metastatic ability [9]: in breast
76 [14-17], hepatic [18] bladder [19] and pancreatic [20] cancers, down-regulation of PFN1 promotes
77 metastatic potential, whereas in renal [21] and gastric [22] cancer metastatic disease, loss of PFN1 reduces
78 metastatic potential.
79 The role of PFN1 in EC has not been investigated to date. PFN1 was identified as potential biomarker for
80 Stage 1 EC by a proteomics screen [23], however this was not validated by another method. We
81 hypothesized that PFN1 expression would be altered in human EC, similar to other epithelial malignancies
82 and contribute to the development of EC. The aim of this study was to quantify PFN1 expression in type 1
83 human endometroid EC and determine the effect of down-regulated PFN1 on HEC1A and AN3CA
84 (endometrial epithelial cancer cell lines) cell adhesion and proliferation. The effect of PFN1 on THP1 pro-
85 inflammatory cytokine gene expression was investigated.
86

87 **Materials and methods**

88 *Patient samples.*

89 This study was approved by the Monash Health Human Research and Ethics Committee (approval no.
90 06014C) and the Victorian Cancer Biobank (Melbourne, Victoria, Australia; project no. 13018). Informed
91 consent was obtained from each participant.
92 The Victorian Cancer Biobank provided RNA from type 1 EC (n=9-10/grade) or benign post-menopausal
93 endometrium (n=10). There was no difference in patient age between the three EC grades (median age
94 of patients GI: 55.0 years, range 34-70; GII: 64 years, range 37-82; GIII: 66.5 years, range 42-82), while age
95 was not available for patients donating benign endometrium. Samples were collected in Melbourne,
96 Victoria, Australia between 2007 and 2014.

97 *Cell lines*

98 Cells were cultured in a 37°C humidified incubator containing 5% CO_2. GI-derived Ishikawa cells were
99 provided by Dr Nishida (Tsukuba University, Tochigi, Japan) in 2014 and cultured in DMEM/F12 medium
100 (Thermo Fisher Scientific, Inc.) supplemented with 10% fetal bovine serum (FBS; Invitrogen; Thermo Fisher
101 Scientific, Inc.). GII-derived HEC1A cells [authenticated by the Monash Health Translation Precinct (MHTP)
102 Medical Genomics Facility in 2016] were cultured in McCoy's medium (Thermo Fisher Scientific, Inc.)
103 supplemented with 10% FCS. GIII-derived AN3CA cells (authenticated by the MHTP Medical Genomics
104 Facility in 2016) were cultured in DMEM/F12 medium supplemented with 10% FBS. THP-1 cells (human
105 monocyte cell line non-adherent; authenticated by MHTP Medical Genomics Facility in 2016) were
106 cultured in RPMI (Thermo Fisher Scientific, Inc) containing 10% FBS and 1% antibiotics (penicillin,
107 streptomycin, amphoceterin B; Gibco), before stimulation towards an adherent, macrophage phenotype
108 by overnight treatment with phorbol 12-myristate 13-acetate (PMA; 12.5ng/ml).

109 *RNA isolation, RT-PCR and qRT-PCR*

110 Total RNA was isolated (TRI Reagent RNA Isolation system, Sigma #T9424) and DNase I treated
111 (DNAfree^{TM}, Ambion) before reverse transcription (Superscript III First-Strand Synthesis System,
112 Invitrogen) according to the manufacturer's protocol as previously described [24], except 0.5µl
113 Superscript III was used for each reverse transcription reaction instead of 1µl.

114 PCR was performed as previously described [25] using GoTaq Green Master Mix (Promega) according to
115 the manufacturer's instructions. Briefly, 1µg cDNA was combined with 2x Master Mix and 10µM primers
116 (sequences shown in Table 1) and performed on a Veriti Thermal Cycler (Applied Biosystems). PCR
117 products were visualized with GelRed Nucleic Acid Stain (Biotium) on a 1.6% agarose gel.

118 qPCR was performed as previously described [10]. Briefly, qPCR analyses were performed on the ABI
119 7500HT fast block real time PCR system (Applied Biosystems, Foster City, CA, USA) in triplicate in 384-well
120 Micro Optical plates (Applied Biosystems) with the Power SYBR green master mix (Applied Biosystems)
121 and 200 nM primers (sequences shown in Table 1).

122 The PCR and qPCR protocol was 95°C for 10 min and 40 cycles of 95°C for 15 s followed by 60°C for 1 min.
123 qPCR relative expression levels were calculated by the comparative cycle threshold method (ΔΔCt), with
124 18S ribosomal RNA serving as the endogenous control for normalization.

125 *Immunohistochemistry*

126 An endometrial cancer mid-density tissue array slide (EMC1502; US Biomax, Inc) was used for PFN1 IHC
127 as previously described for normal endometrial tissue [10]. Briefly, PFN1 (Santa Cruz Biotech #sc-137236;
128 1:400) or negative control antibody (Mouse IgG, Dako) was applied overnight at 4°C, before localization
129 detected by Vectastain ABC Elite kit (Vector) and visualized using diaminobenzidine substrate (DAB,
130 DakoCymation). As no negative control could be included on the array slide a positive and negative control
131 section of proliferative phase endometrium was included in the run. CellSense software quantified DAB
132 staining expressed as intensity per core. To give an intensity score for only the endometrial epithelial cell
133 compartment for each core a blinded scorer semi-quantified epithelial cell staining (0, no staining, to 3,
134 intense staining). Cores with less than ¼ tissue present or completely absent positive staining were
135 excluded from analysis.

136 *Western blotting*

137 Endometrial cancer cell lysates from cells cultured under standard conditions (described above) were
138 assayed for total protein and subjected to Western blotting for PFN1 as previously described [11], except
139 30μg total protein was loaded. Briefly, membranes were blocked using 0.1% Tween, 5% skim milk before
140 primary antibody incubation (PFN1, overnight at 4°C, 1:1000, Santa Cruz Biotechnology sc-137236;
141 GAPDH, 1hr at room temperature, 1:2000, CST #3683S) and PFN1 detected using ECL Plus Detection
142 system (GE Healthcare).

143 *siRNA transfection of PFN1*

144 HEC1A and AN3CA's were transfected using RNAiMAX lipofectamine (Thermo Fisher Scientific, Inc) and
145 ON-TARGETplus SMARTpool siRNA (FC: 1µM; PFN1 (5216): #L-102003-00-0010; scramble control: D-
146 001810-10-05; Dharmacon) according to the manufacturer's instructions. xCELLigence assays and RT-
147 qPCR for transfection efficiency were performed 72h after initial transfection.

148 *xCELLigence real-time adhesion and proliferation assay*

149 The real-time cell analyzer (RTCA) SP xCELLigence instrument (ACEA Biosciences; Agilent Technologies
150 GmbH), was used as previously described [26]. Cells were seeded in E-plate 96 (ACEA Biosciences; Agilent
151 Technologies GmbH) at ~10,000 cells/well in media supplemented with 5% FBS. Plates were monitored
152 every 15min for a total of 72h.

153 *PFN1 treatment for THP1 cytokine production*

154 Adherent THP-1 macrophage cells were treated with recombinant human PFN1 (100µM; Abcam
155 #ab87760) or vehicle control (PBS) for 24 and 48hr before RNA was extracted and RT-qPCR performed as
156 described above.

157 *Statistical Analysis*

158 All statistical analyses were performed on raw data using GraphPad Prism 8.4.3 (GraphPad, SanDiego, CA,
159 US). Data was tested for normality where possible ($n>6$) and paired t-tests, one-way ANOVA (followed by
160 Tukey's multiple comparison test) and two-way-ANOVA (followed by Sidak's multiple comparison test)
161 were used as appropriate: the test used is indicated in the figure legends. All data is presented as
162 mean±SEM. A $p<0.05$ was considered statistically significant.

163 **Results**

164 *Endometrial epithelial PFN1 is down-regulated with increasing cancer grade.*

165 There was no change in *PFN1* mRNA expression (Figure 1A) between benign endometrium or EC of any
166 grade. PFN1 immunolocalized to endometrial epithelial, vascular endothelial and immune cells in normal

167 endometrium (Figure 1B-C) as previously described [10]. Strong PFN1 immunostaining was observed in
168 glandular epithelial cells in normal endometrium and GI EC epithelial cancer cells but PFN1
169 immunostaining was reduced in epithelial cancer cells at GII and GIII (Figure 1C). Strong immunostaining
170 of stromal compartment cells morphologically resembling immune cells was consistently observed in
171 normal and cancerous (GI-III) endometrium although these cells were more common in EC (Figure 1C).
172 PFN1 immunostaining intensity in whole tissue (quantified by CellSense software) was significantly
173 increased in GI EC compared to normal, GI-II and GIII EC (Figure 1D; $F_{4,64}$ 5.580, $p<0.05$). PFN1
174 immunostaining of the endometrial epithelial cancer cellular compartment alone was significantly
175 decreased in GII and GIII EC compared to normal endometrial tissue and GI EC (Figure 1E; $F_{4,74}$ 9.620;
176 $p<0.05$).

177 *Silencing PFN1 significantly increased AN3CA adhesion and proliferation*

178 As the endometrial epithelial cell compartment showed significant loss of PFN1 with increasing cancer
179 grade we determined the functional effect of silencing PFN1 on adhesion and proliferation in endometrial
180 epithelial cancer cell lines. PFN1 mRNA and protein was highly detectable in Ishikawa (GI), HEC1A (GII)
181 and AN3CA (GIII) cell lines and there was no difference in production between the three cell lines (Figure
182 2A&B). As loss of PFN1 was found in GII and GIII EC, we investigated the effect of silencing PFN1 in HEC1A
183 (GII) and AN3CA (GIII) EC cell lines (Figure 2C-E). Silencing PFN1 increased AN3CA cell index (a measure of
184 cell attachment) during the period of cell adhesion (up to 6h; 3-6h $p<0.05$; Figure 2C) and proliferation
185 (up to 72h; 36-72h $p<0.05$; Figure 2D), but had no effect in HEC1A cells. Knockdown efficiency for each
186 cell line is shown in Figure 2E.

187 *PFN1 down-regulated THP1 production of TNFa and IL1b*

188 As PFN1 immunostaining intensity was strong in infiltrating immune cells in EC, we investigated whether
189 PFN1 regulated THP1 cytokine production. Recombinant human PFN1 treatment for 48h significantly
190 reduced THP1 expression of *TNFα* and *IL1β* (Figure 3A&B; p<0.05). No significant effect was seen for *IL12α*.

191 **Discussion**

192 This is the first study to characterize and investigate the function of PFN1 in type 1 EC. We demonstrated
193 that PFN1 production was decreased with increasing EC grade in endometrial epithelial cancer cells. *In*
194 *vitro* experiments indicated that loss of PFN1 could increase EC adhesion and proliferation. We detected
195 PFN1 immunostaining in EC infiltrating immune cells and showed that exogenous PFN1 down-regulated
196 THP1 macrophage production of pro-inflammatory cytokines *TNFa* and *IL1b*.

197 In a previous EC biomarker discovery report, proteomics identified increased PFN1 in Stage 1 EC compared
198 to adjacent peri-cancerous endometrial tissue collected from the same woman [23]. Here we found an
199 increase in PFN1 immunostaining in GI EC (whole tissue) compared to benign endometrium and GI-II and
200 GIII EC, however for the first time we report that PFN1 immunostaining was decreased specifically in
201 epithelial cancer cells from GII-GIII EC. Our immunostaining suggests increased PFN1 in GI EC is likely due
202 to increased immune infiltration into the cancer lesion which begins in GI EC, whereas the reduction in
203 PFN1 protein found in GII and GIII EC is likely due to loss of PFN1 in the epithelial cancer cells. Overall, our
204 data demonstrates the importance in localizing PFN1 expression to individual cells during EC progression.

205 The mechanism leading to altered PFN1 production by GII and GIII endometrial epithelial cancer cells is
206 unknown. Changes to actin networks can alter PFN1 synthesis [9]; given that disruptions to the actin
207 cytoskeleton is a hallmark of cancer cells [14] it is likely that PFN1 down-regulation reflects the
208 transformation of these cells to cancerous cells. Whether the down-regulation of PFN1 in EC is
209 transcriptional or post-transcriptional cannot be determined from this study: gene expression levels did
210 not change with increasing cancer grade, probably due to increasing PNF1 positive immune cell

211 infiltration. miR-182 is a tumor promotor in a number of cancers including EC [27] and breast where it
212 promotes tumorigenesis by down-regulating PFN1 [28]. In other tissues PFN1 regulates multiple pathways
213 which are dysregulated in endometrial cancer, including PI3K, PTEN and p53 [9, 29, 30]. Our observation
214 that the effect of silencing PFN1 was cell line specific may be related to the mutational background of
215 these cells: AN3CA have mutations in *PI3KR1* and *PTEN*, whereas HEC1A has mutations in *KRAS* and *PI3KCA*
216 [31]. Future experiments could be directed towards identifying the mechanism by which PFN1 inhibits cell
217 proliferation in EC cell lines with differing mutational backgrounds.

218 PFN1 has tissue specific pro- or anti-tumerogenic actions [9]. It was demonstrated here that in EC cells,
219 PFN1 may be anti-tumorogenic via its actions to suppress proliferation. Lower PFN1 is also seen in breast
220 cancer where loss of PFN1 has been shown *in vitro* to increase the metastatic potential of epithelial breast
221 cancer cells through increased proliferation, less apoptosis and increased migration and invasion [15-17].
222 PFN1 also impairs proliferation in hepatic [18] and bladder [19] cancer cells. However, breast and bladder
223 cancer xenograft models made with cell lines where PFN1 is silenced show impaired tumorigenesis [14]
224 [19]. This highlights the importance of the tumor microenvironment in tumorigenesis.

225 EC immunostaining presented here suggests that immune cells infiltrating into the EC strongly express
226 PFN1. We have previously shown that endometrial stromal cells do not produce detectable levels of PFN1
227 by immunostaining and that the only cells staining for PFN1 in the stroma are likely leukocytes [10, 11].
228 Whilst we did not perform IHC for immune cell markers in this study, previous research have shown that
229 the EC microenvironment is characterized by infiltration of immune cells which are thought to influence
230 EC progression and patient outcomes [32]. We found that PFN1 treatment down-regulated THP1 pro-
231 inflammatory cytokine expression *in vitro*. Our observation that *TNFα* and *IL1β* expression was not
232 reduced until 48h after PFN1 treatment suggests that PFN1 did not directly regulate *TNFα* and *IL1β* mRNA
233 expression. We have previously demonstrated that *in vitro* PFN1 down-regulates the production of
234 arachidonate 5-lipoxygenase (ALOX5) in THP1 macrophages and human endometrial stomal cells [10].

235 ALOX5 is a lipoxygenase enzyme which converts arachidonic acid to leukotrienes [33] and inhibition of
236 ALOX5 down-regulates the expression of pro-inflammatory cytokines including TNFα [34], IL6 [34] and
237 IL1β [35]. Strong PFN1 staining of cells within the stromal compartment of EC therefore might reflect a
238 pro-tolerance tumor microenvironment: strong PFN1 staining within the stromal compartment of renal
239 and gastric cancer is also observed [21, 22].

240 In conclusion, we have identified that PFN1 is dysregulated in EC and that differential expression of PFN1
241 between endometrial epithelial cancer cells and infiltrating immune cells may play a key role in the
242 tumorigenesis of EC. Loss of PFN1 in GII and GIII endometrial epithelial cancer cells associated with
243 sustained expression of PFN1 by infiltrating immune cells may promote tumorigenesis due to increased
244 EC cell proliferation coupled with a pro-tolerance tumor microenvironment. The role of PFN1 in EC should
245 be confirmed using primary EC cells and *in vivo* mouse models.

246 **Acknowledgements**

247 The authors would like to acknowledge the invaluable contribution of the Doctors for providing tissues
248 and the Victorian Cancer Biobank, in particular Ms Zdenka Prodanovic. We also thank Jeanne Griffin,
249 Katarzyna Rainczuk, Teresa So and Dr Wei Zhou for technical assistance.

250 **Funding**

251 This work was supported by the NHMRC (Australia) [GNT1098332, #550905, #1120300 and #611827],
252 Rebecca L Cooper Medical Research Foundation and the Victorian Government's Operational
253 Infrastructure Support.

254 **References**

255 [1] J. Ferlay, H.R. Shin, F. Bray, D. Forman, C. Mathers, D.M. Parkin, Estimates of worldwide burden of
256 cancer in 2008: GLOBOCAN 2008, Int J Cancer, 127 (2010) 2893-2917.

257 [2] P.T. Soliman, J.C. Oh, K.M. Schmeler, C.C. Sun, B.M. Slomovitz, D.M. Gershenson, T.W. Burke, K.H. Lu,
258 Risk factors for young premenopausal women with endometrial cancer, Obstet Gynecol, 105 (2005) 575-
259 580.
260 [3] O. Bilyk, M. Coatham, M. Jewer, L.-M. Postovit, Epithelial-to-Mesenchymal Transition in the female
261 reproductive tract: from normal function to disease pathology, Frontiers in Oncology, 7 (2017) 145.
262 [4] V. Makker, A.K. Green, R.M. Wenham, D. Mutch, B. Davidson, D.S. Miller, New therapies for
263 advanced, recurrent and metastatic endometrial cancers, Gynecologic oncology research and practice, 4
264 (2017) 19.
265 [5] B. Hong, M. Le Gallo, D.W. Bell, The mutational landscape of endometrial cancer, Curr Opin Genet
266 Dev, 30 (2015) 25-31.
267 [6] A. Di Cristofano, L.H. Ellenson, Endometrial Carcinoma. , The Annual Review of Pathology, 2 (2007)
268 57-85.
269 [7] J.M. Piulats, E. Guerra, M. Gil-Martin, B. Roman-Canal, S. Gatius, R. Sanz-Pamplona, A. Velasco, A.
270 Vidal, X. Matias-Guiu, Molecular approaches for classifying endometrial carcinoma, Gynecologic
271 Oncology, 145 (2017) 200-207.
272 [8] R.C. Arend, B.A. Jones, A. Martinez, P. Goodfellow, Endometrial cancer: Molecular markers and
273 management of advanced stage disease, Gynecologic oncology, (2018).
274 [9] R.J. Davey, P.D.J. Moens, Profilin: many facets of a small protein, Biophysical Reviews, (2020).
275 [10] E.M. Menkhorst, M.L. Van Sinderen, K. Rainczuk, C. Cuman, A. Winship, E. Dimitriadis, Invasive
276 trophoblast promote stromal fibroblast decidualization via Profilin 1 and ALOX5, Scientific Reports, 7
277 (2017) 8690.
278 [11] E.M. Menkhorst, N. Lane, A. Winship, P. Li, J. Yap, K. Meehan, A. Rainczuk, A.N. Stephens, E.
279 Dimitriadis, Decidual-secreted factors alter invasive trophoblast membrane and secreted proteins
280 implying a role for decidual cell regulation of placentation, PLoS ONE, 7 (2012) e31418.
281 [12] C. Théry, M. Boussac, P. Véron, P. Ricciardi-Castagnoli, G. Raposo, J. Garin, S. Amigorena, Proteomic
282 Analysis of Dendritic Cell-Derived Exosomes: A Secreted Subcellular Compartment Distinct from
283 Apoptotic Vesicles, The Journal of Immunology, 166 (2001) 7309-7318.
284 [13] E. Caglayan, G.R. Romeo, K. Kappert, M. Odenthal, M. Südkamp, S.C. Body, S.K. Shernan, D.
285 Hackbusch, M. Vantler, A. Kazlauskas, S. Rosenkranz, Profilin-1 Is Expressed in Human Atherosclerotic
286 Plaques and Induces Atherogenic Effects on Vascular Smooth Muscle Cells, PLoS ONE, 5 (2010) e13608.
287 [14] C. Jiang, Z. Ding, M. Joy, S. Chakraborty, S.H. Kim, R. Bottcher, J. Condeelis, S. Singh, P. Roy, A
288 balanced level of profilin-1 promotes stemness and tumor-initiating potential of breast cancer cells, Cell
289 cycle (Georgetown, Tex.), 16 (2017) 2366-2373.
290 [15] Z. Ding, M. Joy, R. Bhargava, M. Gunsaulus, N. Lakshman, M. Miron-Mendoza, M. Petroll, J.
291 Condeelis, A. Wells, P. Roy, Profilin-1 downregulation has contrasting effects on early vs late steps of
292 breast cancer metastasis, Oncogene, 33 (2014) 2065-2074.
293 [16] Y.H. Bae, Z. Ding, L. Zou, A. Wells, F. Gertler, P. Roy, Loss of profilin-1 expression enhances breast
294 cancer cell motility by Ena/VASP proteins, J Cell Physiol, 219 (2009) 354-364.
295 [17] L. Zou, Z. Ding, P. Roy, Profilin-1 overexpression inhibits proliferation of MDA-MB-231 breast cancer
296 cells partly through p27kip1 upregulation, Journal of Cellular Physiology, 223 (2010) 623-629.
297 [18] Z. Wang, Z. Shi, L. Zhang, H. Zhang, Y. Zhang, Profilin 1, negatively regulated by microRNA-19a-3p,
298 serves as a tumor suppressor in human hepatocellular carcinoma, Pathology - Research and Practice,
299 215 (2019) 499-505.
300 [19] J. Zoidakis, M. Makridakis, P.G. Zerefos, V. Bitsika, S. Esteban, M. Frantzi, K. Stravodimos, N.P.
301 Anagnou, M.G. Roubelakis, M. Sanchez-Carbayo, A. Vlahou, Profilin 1 is a potential biomarker for
302 bladder cancer aggressiveness, Mol Cell Proteomics, 11 (2012) M111.009449-M009111.009449.

303 [20] W. Yao, S. Ji, Y. Qin, J. Yang, J. Xu, B. Zhang, W. Xu, J. Liu, S. Shi, L. Liu, C. Liu, J. Long, Q. Ni, M. Li, X.
304 Yu, Profilin-1 suppresses tumorigenicity in pancreatic cancer through regulation of the SIRT3-HIF1α axis,
305 Molecular cancer, 13 (2014) 187.
306 [21] S. Minamida, M. Iwamura, Y. Kodera, Y. Kawashima, M. Ikeda, H. Okusa, T. Fujita, T. Maeda, S. Baba,
307 Profilin 1 overexpression in renal cell carcinoma, International journal of urology : official journal of the
308 Japanese Urological Association, 18 (2011) 63-71.
309 [22] Y.J. Cheng, Z.X. Zhu, J.S. Zhou, Z.Q. Hu, J.P. Zhang, Q.P. Cai, L.H. Wang, Silencing profilin-1 inhibits
310 gastric cancer progression via integrin β1/focal adhesion kinase pathway modulation, World journal of
311 gastroenterology, 21 (2015) 2323-2335.
312 [23] N. Shan, W. Zhou, S. Zhang, Y. Zhang, Identification of HSPA8 as a candidate biomarker for
313 endometrial carcinoma by using iTRAQ-based proteomic analysis, Onco Targets Ther, 9 (2016) 2169-
314 2179.
315 [24] E. Menkhorst, M. Griffiths, M. Van Sinderen, K. Rainczuk, K. Niven, E. Dimitriadis, Galectin-7 is
316 elevated in endometrioid (type I) endometrial cancer and promotes cell migration, Oncology letters, 16
317 (2018) 4721-4728.
318 [25] A. Winship, J. Correia, J.-G. Zhang, N.A. Nicola, E. Dimitriadis, Leukemia Inhibitory Factor (LIF)
319 Inhibition during Mid-Gestation Impairs Trophoblast Invasion and Spiral Artery Remodelling during
320 Pregnancy in Mice, PloS one, 10 (2015) e0129110-e0129110.
321 [26] M. Van Sinderen, C. Cuman, A. Winship, E. Menkhorst, E. Dimitriadis, The chrondroitin sulfate
322 proteoglycan (CSPG4) regulates human trophoblast function, Placenta, 34 (2013) 907-912.
323 [27] H. Lee, H.J. Choi, C.S. Kang, H.J. Lee, W.S. Lee, C.S. Park, Expression of miRNAs and PTEN in
324 endometrial specimens ranging from histologically normal to hyperplasia and endometrial
325 adenocarcinoma, Modern pathology : an official journal of the United States and Canadian Academy of
326 Pathology, Inc, 25 (2012) 1508-1515.
327 [28] H. Liu, Y. Wang, X. Li, Y.J. Zhang, J. Li, Y.Q. Zheng, M. Liu, X. Song, X.R. Li, Expression and regulatory
328 function of miRNA-182 in triple-negative breast cancer cells through its targeting of profilin 1, Tumour
329 biology : the journal of the International Society for Oncodevelopmental Biology and Medicine, 34
330 (2013) 1713-1722.
331 [29] A.H. Zaidi, N. Raviprakash, R.B. Mokhamatam, P. Gupta, S.K. Manna, Profilin potentiates
332 chemotherapeutic agents mediated cell death via suppression of NF-κB and upregulation of p53,
333 Apoptosis : an international journal on programmed cell death, 21 (2016) 502-513.
334 [30] T. Das, Y.H. Bae, A. Wells, P. Roy, Profilin-1 overexpression upregulates PTEN and suppresses AKT
335 activation in breast cancer cells, J Cell Physiol, 218 (2009) 436-443.
336 [31] B. Weigelt, P.H. Warne, M.B. Lambros, J.S. Reis-Filho, J. Downward, PI3K pathway dependencies in
337 endometrial cancer cell lines, Clinical Cancer Research: an official journal of the American Association for
338 Cancer Research, 19 (2013) 3533-3544.
339 [32] P. Chen, Y. Yang, Y. Zhang, S. Jiang, X. Li, J. Wan, Identification of prognostic immune-related genes
340 in the tumor microenvironment of endometrial cancer, Aging (Albany NY), 12 (2020) 3371-3387.
341 [33] O. Rådmark, O. Werz, D. Steinhilber, B. Samuelsson, 5-Lipoxygenase, a key enzyme for leukotriene
342 biosynthesis in health and disease, Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of
343 Lipids, 1851 (2015) 331-339.
344 [34] H.-C. Lin, T.-H. Lin, M.-Y. Wu, Y.-C. Chiu, C.-H. Tang, M.-J. Hour, H.-C. Liou, H.-J. Tu, R.-S. Yang, W.-M.
345 Fu, 5-Lipoxygenase Inhibitors Attenuate TNF-α-Induced Inflammation in Human Synovial Fibroblasts,
346 PLoS ONE, 9 (2014) e107890.
347 [35] A.P.T. Monteiro, E. Soledade, C.S. Pinheiro, L. Dellatorre-Teixeira, G.P. Oliveira, M.G. Oliveira, M.
348 Peters-Golden, P.R.M. Rocco, C.F. Benjamim, C. Canetti, Pivotal Role of the 5-Lipoxygenase Pathway in
349 Lung Injury after Experimental Sepsis, American Journal of Respiratory Cell and Molecular Biology, 50
350 (2014) 87-95.

351

352 Table 1. Primer sequences.

Primer	Forward 5`-3`	Reverse 5`-3`
18s	GATCCATTGGAGGGCAAGTCT	CCAAGATCCAACTACGAGCTT
IL1β	CAGCCAATCTTCATTGCTCA	TCGGAGATTCGTAGCTGGAT
IL12α	AGAGTCCCGGGAAAGTCCT	TCCAGGAGGACCAGGGTAG
PFN1	GATGGGGAATTTAGCATGGA3	GAAGGGACAGACGAGGTCAG
TNFα	TCAGCCTCTTCTCCTTCCTG	CAGCTTGAGGGTTTGCTACA

353

354 Figure 1. PFN1 production in endometrial cancer (EC). A. *PFN1* mRNA expression by benign post-
355 menopausal endometrium (B) and EC from grades (G) I, II and III (n=9-10/group); B. Positive and negative
356 control for PFN1 IHC. C. PFN1 immunostaining in normal (N) endometrium and EC. D. PFN1
357 immunostaining intensity in normal cycling endometrium (N) and GI, GI-II, GII and GIII EC (one-way
358 ANOVA, n=6-18/group). E. PFN1 immunostaining in endometrial epithelial cells from N endometrium and
359 EC (one-way ANOVA, n=7-20/group). Data presented as mean±SEM; *, P<0.05, significant difference from
360 normal cycling endometrium (N); ^, P<0.05, significant difference from GI endometrial cancer; e, epithelial
361 cell; s, stroma; v, blood vessel; arrow, PFN1 positive cell in stroma, likely immune cell.

362 Figure 2. PFN1 silencing promoted AN3CA cell adhesion and proliferation. A. *PFN1* mRNA expression by
363 EC cell lines Ishikawa (I), HEC1A (H), AN3CA (A). B. PFN1 protein production by EC cell lines. C. PFN1
364 silencing enhanced AN3CA adhesion (two-way ANOVA; n=5/group). D. PFN1 silencing enhanced AN3CA
365 proliferation (two-way ANOVA; n=5/group). E. Confirmation of *PFN1* knockdown by qPCR (paired t-test;
366 n=2-5/group). Data presented as mean±SEM; *,P<0.05; Scr, scramble.

367 Figure 3. PFN1 treatment (100µM) impaired THP1 macrophage pro-inflammatory cytokine production. A.
368 *TNFα* expression (paired t-test; n=6/group); B. *IL1β* expression (paired t-test, n=5-6/group); C. *IL12α*
369 expression (paired t-test, n=3/group). Data presented as mean±SEM; *,P<0.05; Con, control.

Figure

Figure 1

A

10
8
6
4
2
0
ΔΔCt *PFN1/18s*
B GI GII GIII
Endometrial cancer grade

B

Positive
50μm
Negative

C

Normal
s
e
50μm
s
v
e
50μm

Grade I
e
s
s
e

Grade II
s
e
e
s

Grade III
s
e
e
s

D

1.0
0.8
0.6
0.4
0.2
0.0
PFN1 staining Intensity
^
^
^
N GI GI-II GII GIII
Endometrial cancer grade

E

2.5
2.0
1.5
1.0
0.5
0.0
Epithelial PFN1 staining intensity
*
*^
*^
N GI GI-II GII GIII
Endometrial cancer grade

Figure 2

A I H A
PFN1
18S

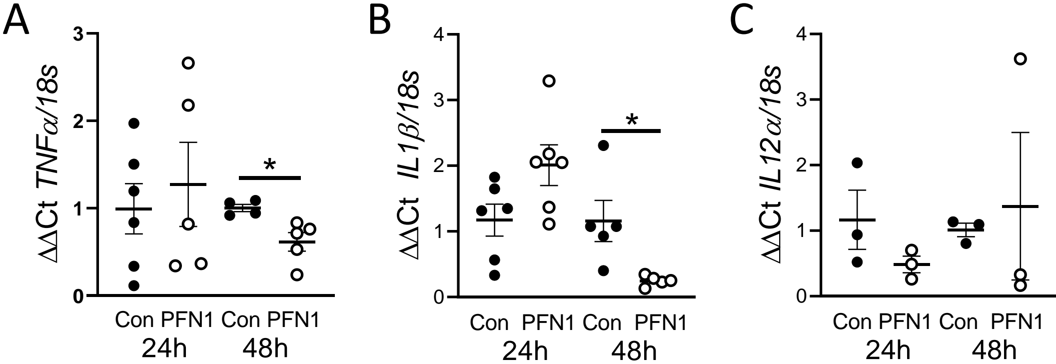
B I H A
PFN1
GAPDH
PFN1/GAPDH
I H A

C Adhesion
D Proliferation
E Knockdown

HEC1A
siRNA treatment
Scramble
PFN1
Cell Index
Time (hours)
ΔΔCt PFN1/18s
Scr PFN1
siRNA treatment

AN3CA
Cell index
Time (hours)
Cell Index
Time (hours)
ΔΔCt PFN1/18s
Scr PFN1
siRNA treatment

Figure 3



Biochemical and Biophysical Research Communications

Declaration of competing interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Please note that all *Biochemical and Biophysical Research Communications* authors are required to report the following potential conflicts of interest with each submission. If applicable to your manuscript, please provide the necessary declaration in the box above.

(1) All third-party financial support for the work in the submitted manuscript.
(2) All financial relationships with any entities that could be viewed as relevant to the general area of the submitted manuscript.
(3) All sources of revenue with relevance to the submitted work who made payments to you, or to your institution on your behalf, in the 36 months prior to submission.
(4) Any other interactions with the sponsor of outside of the submitted work should also be reported.
(5) Any relevant patents or copyrights (planned, pending, or issued).
(6) Any other relationships or affiliations that may be perceived by readers to have influenced, or give the appearance of potentially influencing, what you wrote in the submitted work. As a general guideline, it is usually better to disclose a relationship than not.