

Enhanced Cellular Responses and Distinct Gene Profiles  
in Human Fetoplacental Artery Endothelial Cells under Chronic Low Oxygen

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**Supplemental Methods**

*CD31 Analysis and FACS Sorting*

The cells were gently pipetted after trypsin treatment to dissociate cells into single cell suspension. The cells were then incubated with a mouse monoclonal anti-human CD31 antibody conjugated with R-phycoerythrin (PE) (1  $\mu\text{g}/10^7$  cells, Pharmingen, San Diego, CA) for 30 min at 4 °C. After washing, the cells were resuspended in PBS. Pre-immune mouse IgG conjugated with PE was used in parallel as a negative control. The cells were then sorted with propidium iodide to exclude dead cells on a high-speed Vantage SE sorting cytometer (BD Biosciences, San Jose, CA) with CellQuest and/or the DiVa software at the Carbone Cancer Center, the University of Wisconsin-Madison.

*Ac-LDL Uptake Assay*

The cells were incubated with Ac-LDL labeled with 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI-Ac-LDL, 10  $\mu\text{g}/\text{mL}$ , Biomedical Technologies, Stoughton, MA) for 4 hr at 37°C, fixed in 4% formaldehyde, and examined under a phase-contrast and fluorescent microscope. Images were taken under a Nikon TE2000U inverted microscope connected to a Spot Insight QE CCD camera using the SPOT advanced image analysis software. The cells exhibiting uptake of LDL were counted. The cells incubated without DiI-Ac-LDL were served as negative controls.

*Capillary-Like Tube Formation Assay*

Growth factor-reduced Matrigel matrix (300  $\mu\text{L}$ ; BD Biosciences) was applied to 24-well plate and allowed to polymerize for 30 min at 37 °C. The cells were seeded ( $8 \times 10^4$  cells/well) and incubated under 21% or 3% O<sub>2</sub> for 2 and 24 hr. The capillary-like tube structures were recorded under the microscope.

Table S1. Primers used for qPCR analysis.

Gene Symbol	Probe Sets	Forward Primer	Reverse Primer
<i>CLDN10</i>	205328_at	TTGATCCTCTCTTTGTTGAGCA	AAGCAAAATATGACACCACCAA
<i>GIPC2</i>	219970_at	ATGGAAAATGCTGTGTGCTG	CAGCTTCTGTGCAGATTTCAA
<i>ADAMTS1</i>	222486_s_at	TGAGGCAAGGCAAAGTGAG	ACTGGCAAGATACGCTGGAT
<i>HES1</i>	203395_s_at	TTACGGCGGACTCCATGT	AGAGGTGGGTTGGGGAGT
<i>VEGFC</i>	209946_at	AAGTCCACAGAAATGCTTGTTAAA	TCGTACATGGCCGTCTGTAA
<i>HS6ST2</i>	1552767_a_at	ATTGCTGTGGTGTGCATGAT	AAGCTACACCAATGGACAGATG
<i>TFEC</i>	206715_at	TGAAAACTGATAGAAGCAACAGAA	CGCCCTCAATAATTCATTAACA
<i>VEGFA</i>	210512_s_at	TGCAGATTATGCGGATCAAACC	TGCATTACATTTGTTGTGCTGTAG
<i>AK3L1</i>	204348_s_at	GTCCCAGGCTTTCTGGTGT	GTGGACACAGTCACTCAAGACC
<i>ACTB</i>	200801_x_at	CATTCCAAATATGAGATGCATTG	TGCTATCACCTCCCCTGTGT
<i>TBP</i>	203135_at	CATACCGTGCTGCTATCTGG	TCCCTCAAACCAACTTGTC

Table S2. Antibodies used for Western-blot analysis.

	Antibody	Vendor	Catalog Number	Dilution
1	$\beta$ -actin	Ambion	AM4302	1:10000
2	BNIP3	Abcam	ab10433	1:1000
3	CDCP1	Abcam	ab1377	1:1000
4	FGFR1	Invitrogen	37-9200	1:1000
5	FGFR2	Cell Signaling Technology	3116	1:500
6	SLC2A3	Abcam	ab15311	1:500
7	HIF-1 $\alpha$	BD Bioscience	610959	1:500
8	HIF-2 $\alpha$	Novus	NB100-122	1:500
9	NOS3	BD Bioscience	610297	1:1000
10	VEGFR1	Millipore	05-696	1:1000
11	VEGFR2	Cell Signaling Technology	2479	1:1000

Table S3. PCN-Induced DE Genes with fold changes &lt; 2 in HUAECs.

#	Gene Symbol /Probe sets	Gene Name	Fold Increase
1	<i>ANKRD37</i>	ankyrin repeat domain 37	1.9
2	<i>C1orf21</i>	chromosome 1 open reading frame 21	1.9
3	<i>C9orf64</i>	chromosome 9 open reading frame 64	1.9
4	<i>CEBPD</i>	CCAAT/enhancer binding protein (C/EBP), delta	1.9
5	<i>COBLL1</i>	COBL-like 1	1.9
6	<i>SEPP1</i>	selenoprotein P, plasma, 1	1.9
7	<i>AEBP1</i>	AE binding protein 1	1.8
8	<i>ARL10</i>	ADP-ribosylation factor-like 10	1.8
9	<i>LOX</i>	lysyl oxidase	1.8
10	<i>PDK1</i>	pyruvate dehydrogenase kinase, isozyme 1	1.8
11	<i>REC8</i>	REC8 homolog (yeast)	1.8
12	<i>SEC16B</i>	SEC16 homolog B ( <i>S. cerevisiae</i> )	1.8
13	<i>SSX2IP</i>	synovial sarcoma, X breakpoint 2 interacting protein	1.8
14	<i>DNAJA4</i>	DnaJ (Hsp40) homolog, subfamily A, member 4	1.7
15	<i>KLF11</i>	Kruppel-like factor 11	1.7
16	<i>NEFH</i>	neurofilament, heavy polypeptide 200kDa	1.7
17	<i>SLC2A3</i>	solute carrier family 2 (facilitated glucose transporter), member 3	1.7
18	<i>TXNIP</i>	thioredoxin interacting protein	1.7
19	<i>PLIN2</i>	perilipin 2	1.6
20	<i>ADPRH</i>	ADP-ribosylarginine hydrolase	1.6
21	<i>ANKRD41</i>	ankyrin repeat domain 41	1.6
22	<i>C2orf55</i>	chromosome 2 open reading frame 55	1.6
23	<i>HK2</i>	hexokinase 2	1.6
24	<i>OR2A7</i>	olfactory receptor, family 2, subfamily A, member 7	1.6
25	<i>STX11</i>	syntaxin 11	1.6
26	<i>TRIM14</i>	tripartite motif-containing 14	1.6
27	<i>TUBA4A</i>	tubulin, alpha 4a	1.6
28	<i>VEGFA</i>	vascular endothelial growth factor A	1.6
29	<i>241835_at</i>	N.A.	1.5
30	<i>242582_at</i>	N.A.	1.5
31	<i>FAM115C</i>	family with sequence similarity 115, member C	1.5
32	<i>FOLH1</i>	folate hydrolase (prostate-specific membrane antigen) 1	1.5
33	<i>PION</i>	pigeon homolog ( <i>Drosophila</i> )	1.5
34	<i>SERTAD2</i>	SERTA domain containing 2	1.5
35	<i>SHISA3</i>	shisa homolog 3 ( <i>Xenopus laevis</i> )	1.5
36	<i>DNMT3A</i>	DNA (cytosine-5-)-methyltransferase 3 alpha	1.4
37	<i>EZR</i>	ezrin	1.4
38	<i>FAM108C1</i>	family with sequence similarity 108, member C1	1.4
39	<i>MCCC2</i>	methylcrotonoyl-Coenzyme A carboxylase 2 (beta)	1.4
40	<i>MET</i>	met proto-oncogene (hepatocyte growth factor receptor)	1.4
41	<i>OSBPL3</i>	oxysterol binding protein-like 3	1.4
42	<i>FUT11</i>	fucosyltransferase 11 (alpha (1,3) fucosyltransferase)	1.3

#	Gene Symbol /Probe sets	Gene Name	Fold Decrease
1	<i>C6orf138</i>	chromosome 6 open reading frame 138	-1.9
2	<i>GZMA</i>	granzyme A (granzyme 1, cytotoxic T-lymphocyte-associated serine esterase 3)	-1.9
3	<i>228740_at</i>	N.A.	-1.9
4	<i>RBM24</i>	RNA binding motif protein 24	-1.9
5	<i>BCL2</i>	B-cell CLL/lymphoma 2	-1.8
6	<i>DLC1</i>	deleted in liver cancer 1	-1.8
7	<i>MLF1</i>	myeloid leukemia factor 1	-1.8
8	<i>FBXO32</i>	F-box protein 32	-1.8
9	<i>LOC100505702</i>	uncharacterized LOC100505702	-1.8
10	<i>PAPPA</i>	pregnancy-associated plasma protein A, pappalysin 1	-1.8
11	<i>PARD6G</i>	par-6 partitioning defective 6 homolog gamma ( <i>C. elegans</i> )	-1.8
12	<i>PPAPDC1A</i>	phosphatidic acid phosphatase type 2 domain containing 1A	-1.8
13	<i>SELM</i>	selenoprotein M	-1.8
14	<i>UST</i>	uronyl-2-sulfotransferase	-1.8
15	<i>ADORA3</i>	adenosine A3 receptor	-1.7
16	<i>BPGM</i>	2,3-bisphosphoglycerate mutase	-1.7
17	<i>BVES</i>	blood vessel epicardial substance	-1.7
18	<i>C1orf187</i>	chromosome 1 open reading frame 187	-1.7
19	<i>HS6ST2</i>	heparan sulfate 6-O-sulfotransferase 2	-1.7
20	<i>ITM2C</i>	integral membrane protein 2C	-1.7
21	<i>230537_at</i>	N.A.	-1.7
22	<i>RELN</i>	reelin	-1.7
23	<i>SMPDL3A</i>	sphingomyelinphosphodiesterase, acid-like 3A	-1.7
24	<i>SPG3A</i>	spastic paraplegia 3A (autosomal dominant)	-1.7
25	<i>TMOD2</i>	tropomodulin 2 (neuronal)	-1.7
26	<i>C4orf34</i>	chromosome 4 open reading frame 34	-1.6
27	<i>CEP135</i>	centrosomal protein 135kDa	-1.6
28	<i>CREG1</i>	cellular repressor of E1A-stimulated genes 1	-1.6
29	<i>EDN1</i>	endothelin 1	-1.6
30	<i>GRAP</i>	GRB2-related adaptor protein	-1.6
31	<i>HES1</i>	hairy and enhancer of split 1, ( <i>Drosophila</i> )	-1.6
32	<i>HNMT</i>	histamine N-methyltransferase	-1.6
33	<i>MFAP3L</i>	microfibrillar-associated protein 3-like	-1.6
34	<i>MOV10L1</i>	Mov1011, Moloney leukemia virus 10-like 1, homolog (mouse)	-1.6
35	<i>SMARCD3</i>	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily d, member 3	-1.6
36	<i>TMEM112</i>	transmembrane protein 112	-1.6
37	<i>CHST11</i>	carbohydrate (chondroitin 4) sulfotransferase 11	-1.5
38	<i>CORO1A</i>	coronin, actin binding protein, 1A	-1.5
39	<i>FZD3</i>	frizzled homolog 3 ( <i>Drosophila</i> )	-1.9
40	<i>IDS</i>	iduronate 2-sulfatase (Hunter syndrome)	-1.5
41	<i>KCNMB4</i>	potassium large conductance calcium-activated channel,	-1.5

		subfamily M, beta member 4	
42	<i>SLC16A2</i>	solute carrier family 16, member 2 (monocarboxylic acid transporter 8)	-1.5
43	<i>SPTA1</i>	spectrin, alpha, erythrocytic 1 (elliptocytosis 2)	-1.5
44	<i>TANK</i>	TRAF family member-associated NFkB activator	-1.5
45	<i>TMEM11</i>	transmembrane protein 11	-1.5
46	<i>TNFRSF21</i>	tumor necrosis factor receptor superfamily, member 21	-1.5
47	<i>ULBP2</i>	UL16 binding protein 2	-1.5
48	<i>C15orf52</i>	chromosome 15 open reading frame 52	-1.4
49	<i>CAMK2N1</i>	calcium/calmodulin-dependent protein kinase II inhibitor 1	-1.4
50	<i>CYFIP2</i>	cytoplasmic FMR1 interacting protein 2	-1.4
51	<i>MYO10</i>	myosin X	-1.4
52	<i>CAMK2N2</i>	calcium/calmodulin-dependent protein kinase II inhibitor 2	-1.3
53	<i>NPL</i>	N-acetylneuraminase pyruvate lyase (dihydrodipicolinate synthase)	-1.3

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*Note:* The fold change was the mean of fold change of probe sets. N.A.= no annotation.

Table S4. IPA of cellular functions for PCN-induced DE genes in HUAECs.

Functions Annotation	<i>p</i> -Value	Activation z-score
<i>A. Cellular Movement</i>		
Cell movement of ovarian cancer cell lines	8.8E-06	0.603
Cell movement of cancer cells	3.1E-05	-0.026
Invasion of tumor cell lines	3.1E-05	-0.065
Cell movement	1.0E-04	-0.137
Cell movement of tumor cell lines	1.2E-03	-0.227
Invasion of tumor cells	1.8E-04	-0.243
Invasion of cancer cells	5.1E-05	-0.283
Cell movement of breast cell lines	1.3E-03	-0.283
Invasion of ovarian cancer cell lines	1.7E-07	-0.896
Migration of tumor cell lines	2.9E-03	-1.069
Migration of cells	3.7E-04	-1.242
<i>B. Cellular Development</i>		
Proliferation of ovarian cancer cell lines	1.4E-03	1.109
Adipogenesis of cells	4.4E-03	0.975
Proliferation of cancer cells	7.3E-03	0.530
Differentiation of osteoblasts	1.7E-03	0.339
Proliferation of hematopoietic progenitor cells	6.8E-04	0.103
Proliferation of tumor cell lines	1.4E-03	-0.083
Proliferation of blood cells	1.5E-05	-0.185
Proliferation of bone marrow cells	4.0E-03	-0.391
Proliferation of immune cells	5.8E-04	-0.703
Proliferation of stem cells	6.1E-03	-0.762
Differentiation of tumor cell lines	4.5E-03	-1.103
Proliferation of lymphocytes	1.7E-03	-1.248
Differentiation of cells	2.0E-03	-1.399
Proliferation of melanoma cell lines	4.9E-05	-1.408
Proliferation of T lymphocytes	5.9E-03	-1.464
<i>C. Cellular Growth and Proliferation</i>		
Proliferation of ovarian cancer cell lines	1.4E-03	1.109
Proliferation of cancer cells	7.3E-03	0.530
Colony formation of tumor cell lines	4.3E-03	0.479
Colony formation of cells	2.3E-03	0.178
Proliferation of hematopoietic progenitor cells	6.8E-04	0.103
Proliferation of tumor cell lines	1.4E-03	-0.083
Proliferation of blood cells	1.5E-05	-0.185
Proliferation of cells	5.8E-05	-0.250
Proliferation of bone marrow cells	4.0E-03	-0.391
Proliferation of immune cells	5.8E-04	-0.703
Proliferation of stem cells	6.1E-03	-0.762
Stimulation of cells	5.6E-04	-0.829
Formation of hematopoietic progenitor cells	4.6E-05	-0.927
Stimulation of lymphocytes	2.0E-04	-1.000

Formation of blood cells	1.1E-03	-1.029
Formation of osteoclasts	5.0E-03	-1.121
Colony formation of blood cells	6.0E-03	-1.231
Proliferation of lymphocytes	1.7E-03	-1.248
Proliferation of melanoma cell lines	4.9E-05	-1.408
Proliferation of T lymphocytes	5.9E-03	-1.464
Formation of cells	1.1E-03	-1.523
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<i>D. Cell Death and Survival</i>		
Cell viability	3.8E-04	2.015
Cell survival	4.5E-04	1.629
Cell death of neuroglia	2.3E-03	1.166
Apoptosis of muscle cell lines	7.5E-04	1.117
Cell viability of kidney cell lines	1.3E-03	1.067
Cell viability of tumor cells	5.2E-03	0.555
Apoptosis of muscle cells	4.7E-03	0.431
Cell viability of breast cancer cell lines	3.7E-03	0.394
Cell death of tumor cells	2.5E-03	0.378
Apoptosis of embryonic cells	5.4E-03	0.371
Apoptosis of neuroglia	3.3E-03	0.333
Cell death of muscle cells	5.7E-03	0.111
Cell death	3.1E-05	-0.252
Cell viability of neurons	5.5E-03	-0.272
Apoptosis of cardiomyocytes	2.4E-03	-0.339
Necrosis	6.2E-04	-0.404
Apoptosis of tumor cells	7.2E-03	-0.422
Apoptosis	2.8E-04	-0.452
Cell death of cardiomyocytes	1.0E-03	-0.692
Apoptosis of tumor cell lines	4.6E-04	-1.849
Cell death of tumor cell lines	2.7E-03	-2.236
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<i>E. Cell Morphology</i>		
Sprouting	6.2E-05	-1.274
Outgrowth of neurites	1.7E-03	-0.293
Morphology of cells	1.0E-03	1.446

*Note:* A positive or negative z-score implies potential activation or inhibition of the pathway.