Identification of target genes for a prolactin family paralog in mouse decidua

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Abstract

Prolactin family 8, subfamily a, member 2 (PRL8A2; also called decidual prolactin-related protein; dPRP) is a member of the expanded prolactin family. PRL8A2 is expressed in the uterine decidua and contributes to pregnancy-dependent adaptations to hypoxia. The purpose of this study was to identify gene targets for PRL8A2 action within the uteroplacental compartment. Affymetrix DNA microarray analysis was performed for RNA samples from WT and *Prl8a2* null tissues. Validation of the DNA microarray was performed using quantitative RT-PCR. Nine genes were confirmed with decreased expression in *Prl8a2* null tissues (e.g. *Klk7*, *Rimklb*, *Arhgef6*, *Calm4*, *Spr2h*, *Prl4a1*, *Ccl27*, *Lipg*, and *Htra3*). These include potential decidual, endothelial and trophoblast cell targets positively regulated by PRL8A2. A significant upregulation of *Derl3*, *Herpud1*, *Crel2*, *Hsp90b1*, *Ddit3* and *Hspa5* was identified in *Prl8a2* null tissues, reflecting an increased endoplasmic reticulum (ER) stress response. ER stress genes were prominently expressed in the uterine decidua. We propose that PRL8A2 is a mediator of progesterone-dependent modulation of intrauterine responses to physiological stressors.

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Introduction

The mouse possesses an expanded prolactin (PRL) gene family that encodes hormones/cytokines (Wiemers *et al.* 2003, Soares *et al.* 2007). In some species the expansion was robust such as occurred in the mouse, rat, guinea pig and cow (Wiemers *et al.* 2003, Alam *et al.* 2006, 2010, Ushizawa & Hashizume 2006), whereas evidence for an expansion in other species such as the human and dog is lacking (Cooke & Liebhaber 1995, Lindblad-Toh *et al.* 2005). These hormones and cytokines are associated with pregnancy and are produced by the anterior pituitary, uterine decidua and/or trophoblast cells (Soares 2004). The biological activities of PRL are well described and include profound effects on the reproductive axis and lactation (Horseman *et al.* 1997, Bole-Feysot *et al.* 1998, Horseman & Gregerson 2014); however, the actions of the remaining PRL family paralogs are less well appreciated. Roles for these PRL related proteins in regulating blood vessel and hematopoietic cell development have been demonstrated (Jackson *et al.* 1994, Lin & Linzer 1999, Bittorf *et al.* 2000). Based on mouse mutagenesis experiments, the biological activities of at least some expanded PRL family paralogs include modulation of uteroplacental

adaptations to physiological stressors (Ain *et al.* 2004, Alam *et al.* 2007, Soares *et al.* 2007). PRL also participates in homeostatic responses to stress (Dorshkind & Horseman 2001).

Hemochorial placentation is associated with differentiation of uterine stromal cells into epithelial-like cells called decidual cells possessing extensive secretory capabilities and essential roles in the establishment and maintenance of pregnancy (Aplin 2000, Gellersen *et al.* 2007, Herington & Bany 2009, Teklenburg *et al.* 2010a,b). Decidual cells effectively create an environment within the uterus compatible with development of the placenta and fetus. Among the factors secreted by decidual cells are members of the PRL family (Orwig *et al.* 1997a, Jabbour & Critchley 2001). Human decidual cells produce PRL, while the mouse and rat produce PRL and an additional three PRL family paralogs (Soares 2004, Soares *et al.* 2007).

Biological roles for decidual PRL family hormones/cytokines are not well understood (Jabbour & Critchley 2001). Among the decidual PRL family paralogs in the mouse and rat is a protein referred to as PRL family 8, subfamily a, member 2 (PRL8A2; also referred to as decidual PRL-related protein dPRP; Roby *et al.* 1993).

PRL8A2 is abundantly expressed in the uterine decidua (Roby *et al.* 1993, Gu *et al.* 1994, Rasmussen *et al.* 1996, 1997, Lin *et al.* 1997, Orwig *et al.* 1997a,b,c, Orwig & Soares 1999, Bany & Cross 2006, Alam *et al.* 2008), binds to heparin and although it is structurally similar to PRL it does not appear to signal through the PRL receptor (Rasmussen *et al.* 1996, Wang *et al.* 2000, Alam *et al.* 2008). PRL8A2 deficiency interferes with pregnancy-dependent adaptations to hypoxia resulting in pregnancy failure (Alam *et al.* 2007).

The purpose of this study was to identify candidate targets for PRL8A2 action within the uteroplacental compartment.

Materials and methods

Animals and tissue preparation

C57BL/6 mice were obtained from Jackson Laboratories (Bar Harbor, ME, USA). Mice were housed in an environmentally controlled facility, with lights on from 0600 to 2000 h, and allowed free access to food and water. Timed matings of animals were conducted by placing females with fertile males. The day when a seminal plug was found in the vagina of female mice was designated as day 0.5 of pregnancy. Placentation sites, including uterus, decidual and placental tissues, were dissected from pregnant animals. Harvested tissues were snap-frozen in liquid nitrogen for RNA and protein analyses. For *in situ* hybridization analyses, tissues were frozen in dry ice-cooled heptane. All tissue samples were stored at -80°C until used. Protocols for the preceding procedures have been described (Ain *et al.* 2006, Deb *et al.* 2006, Alam *et al.* 2007, 2008). The University of Kansas Medical Center Animal Care and Use Committee approved all procedures for handling and experimentation with rodents.

DNA microarray

WT and *Prl8a2* null mice were mated and sacrificed on gestation day 7.5. Decidual-placental-embryonic tissues were dissected from implantation sites and homogenized. Total RNA

was extracted using TRIzol reagent according to the manufacturer's protocol (Invitrogen). RNA extractions were pooled to form three groups of three for each group in nuclease-free water at a concentration of $1.0\text{ }\mu\text{g}/\mu\text{l}$. RNA samples were hybridized to the Affymetrix 430 2.0 DNA microarray chip using the GeneChip Hybridization Oven 640 (Affymetrix, Santa Clara, CA, USA). Washing and staining of the hybridized chips were conducted using the GeneChip Fluidics Station 450 (Affymetrix). Chips were scanned using the Affymetrix GeneChip Scanner 3000 (Affymetrix) with autoloader by the KUMC Biotechnology Support Facility. Hybridization signals were normalized with internal controls. Expression data sets were analyzed using the expression analysis software GeneSpring 7.0 and R Statistics Software (<http://www.r-project.org/>) with BioConductor Software (<http://www.bioconductor.org/>) packages. The RMA method from the BioConductor Software was used for background correction, normalization and summarization of the DNA microarray data. Statistical comparisons of expression values between two groups were determined with a moderated *t*-test. Pathway analysis was performed with AltAnalyze (<http://altanalyze.org>) and PathVisio (<http://www.pathvisio.org>).

Quantitative RT-PCR

cDNAs were synthesized with total RNA ($1\text{ }\mu\text{g}$) from each sample using M-MLV reverse transcriptase (Invitrogen), diluted five times with water and subjected to quantitative RT-PCR (qRT-PCR) to quantify mRNA levels of the genes identified from the DNA microarray. Primers were designed using Primer Express 2.0 (Applied Biosystems). Primer sequences can be found in Table 1. Real-time PCR amplification of cDNAs was carried out in a reaction mixture ($10\text{ }\mu\text{l}$) containing SYBR GREEN PCR Master Mix (Applied Biosystems) and primers (600 nM each). Amplification and fluorescence detection were carried out using the ABI Prism 7500 Real Time PCR System (Applied Biosystems). Cycling conditions included an initial hold step (95°C for 10 min) and 40 cycles of a two-step PCR (92°C for 15 s, then 60°C for 1 min), followed by a dissociation step (95°C for 15 s, 60°C for 15 s, and then 95°C for 15 s). qRT-PCR for each query mRNA was validated, including

Table 1 Primer sequences for transcripts regulated by PRL8A2.

Gene	GenBank accession no.	Forward primer	Reverse primer
<i>Rimkb</i>	NM_027664	TGAAGGCCAAATGTTGTGAA	TCTCCACTGATCCGAAGACC
<i>Klk7</i>	NM_011872	TCTGGCTCCTTCCCTGATA	GGTGGCAGCCTTCTTTACAT
<i>Ccl27</i>	NM_001048179	GACTGTACCTCCAGGCTGT	CTTTCCCTTGGCGTTCTAA
<i>Calm4</i>	NM_020036	CAGAGATGTCTCAGCGGTTT	GTTCTCCGACGCTGATATGG
<i>Prl4a1</i>	NM_011165	GGAGACCATAGAGAAGATT	GCAAGAGTTCCAATTCAGA
<i>Lipg</i>	NM_010720	CCAAACCAAAAACCTGCTTG	CGCCGGGAAGTAACAATAGA
<i>Htra3</i>	NM_001042615	CCGATGTGGTGGAGAAGATT	ACTGGACAGCGGCACATT
<i>Sprr2h</i>	NM_011474	ACACTTGGTACTCAAGTCTT	AAGGCTGCTTGCACTGCT
<i>Arhgef8</i>	NM_152801	TCCCCTAAGGCTATCAAAGGA	GGCATATCTTTTTCAGTGTCC
<i>Derl3</i>	NM_024440	GGGATTCGGCTCTTTTTCAA	CATGAAAACGAAGTCAGCCTT
<i>Herpud1</i>	NM_022331	CCTCAGCATCCTTTACTTCT	CTCTGTCTGAACGGAAACCA
<i>Crelid2</i>	NM_029720	ACTGCACAGACGGCTTCTTC	CTTGACACAGACAGGCTTCT
<i>Hsp90b1</i>	NM_011631	ATGGCACAGTGAAGAGGAC	TGCGTTTAACCCATCCAAC
<i>Ddit3</i>	NM_007837	CACCTATATCTCATCCCCA	GGATGTGCGTGTGACCTCT
<i>Hspa5</i>	NM_0011634	TGCAGCAGGACATCAAGTTC	TTTCTTCTGGGGCAAATGTC
<i>18S</i>	NR_003278	GCAATTATTCCTCATGAACG	GGCCTCACTAAACCATCCAA

determining amplification efficiencies and co-linearity of the query mRNAs and 18S rRNA. The comparative CT method was used for relative quantification of the amount of mRNA for each sample normalized to 18S RNA.

In situ hybridization

The localization of mRNAs within tissues was performed as described previously (Ain *et al.* 2003, Wiemers *et al.* 2003). Cryosections (10 µm) of tissues were prepared and stored at –80 °C until used. Plasmids containing cDNAs for mouse *Rimklb*, *Derl3*, *Hspa5* and *Hsp90b1* were used as templates to synthesize sense and antisense digoxigenin labeled riboprobes according to the manufacturer's instructions (Roche Molecular Biochemicals). Images were captured using a Leica MZFIII stereomicroscope (Leica Microsystems GmbH, Wetzlar, Germany) or a Nikon Eclipse 55i microscope (Nikon Instruments, Inc., Melville, NY, USA), both equipped with Leica CCD cameras (Leica).

Statistical analysis

Statistical analyses were performed using the R Statistical Software (<http://www.r-project.org>). Statistical comparisons between two means were determined with Student's *t*-test or Welch's *t*-test, depending on the homogeneity of variances.

Results

Mice possessing null mutations at the *Prl8a2* locus reproduce within the normal range but unlike WT mice do not effectively adapt when exposed to hypoxic conditions during pregnancy (Alam *et al.* 2007). This mutant mouse model was used as a tool to identify downstream actions of PRL8A2 signaling. We used DNA microarray analysis to examine the consequences of PRL8A2 deficiency on gene expression at gestation day 7.5. Gestation day 7.5 is associated with robust *Prl8a2* expression and represents a pivotal time point in

Table 2 List of transcripts downregulated (greater than or equal to twofold) in implantation sites of the PRL8A2 deficient mouse.

Gene name	Symbol	GenBank accession no.	Function	Ratio (null/WT)
Prolactin family 8, subfamily A, member 2	<i>Prl8a2</i>	NM_010088	Hormone/cytokine	0.00
Ribosomal modification protein rimK-like family member B	<i>Rimklb</i>	AV271892	ATP binding, amino acid ligase activity, glutathione synthase activity	0.10
Kallikrein related peptidase 7	<i>Klk7</i>	BB283507	Trypsin-like serine protease	0.22
Midline 1	<i>Mid1</i>	BG073178	Microtubule associated	0.25
Chemokine (C-C motif) ligand 27a	<i>Ccl27a</i>	NM_011336	Chemokine, leukocyte recruitment	0.28
Predicted gene, EG633640	<i>EG633640</i>	BG068672	Unknown	0.28
Proline rich 9	<i>A030004J04Rik</i>	BB150166	Unknown	0.30
Orosomucoid 1	<i>Orm1</i>	BE628912	Transporter activity/immune-related	0.30
Calmodulin 4	<i>Calm4</i>	NM_020036	Calcium signaling	0.35
Porcupine homolog	<i>Porcn</i>	AB036749	Wnt signaling pathway	0.35
Predicted gene 9780	<i>MGI:3710532</i>	AI508243	Unknown	0.36
Expressed sequence tag	–	AV271189	Unknown	0.40
Orosomucoid 2	<i>Orm2</i>	NM_011016	Transporter activity/immune-related	0.40
Cellular retinoic acid binding protein	<i>Crabp2</i>	BC018397	Retinoic acid transport	0.40
Expressed sequence tag	–	BG083989	Unknown	0.40
Lipase, endothelial	<i>Lipg</i>	BC020991	Lipid metabolism	0.41
A disintegrin-like and metalloproteinase with thrombospondin type 1 motif, 5	<i>Adamts5</i>	BB658835	Integrin-mediated signaling, metalloproteinase	0.41
Prolactin family 4, subfamily A, member 1	<i>Prl4a1</i>	NM_011165	Hormone/cytokine	0.42
HtrA serine peptidase 3	<i>Htra3</i>	NM_030127	Serine protease	0.43
Expressed sequence tag	–	BM115786	Unknown	0.43
Small proline-rich protein 2H	<i>Spr2h</i>	NM_011474	Epithelial barrier	0.43
Neuromedin U	<i>Nmu</i>	NM_019515	Neuropeptide signaling	0.43
PR domain containing 16	<i>Prdm16</i>	BB356786	Transcription coregulator	0.44
Endogenous retroviral sequence 3	<i>Erv3</i>	AK005451	Unknown	0.44
Carcinoembryonic antigen-related cell adhesion molecule 9	<i>Ceacam9</i>	NM_011927	Immune-related	0.44
Expressed sequence tag	–	AU067772	Unknown	0.45
Expressed sequence tag	–	BB712583	Unknown	0.45
Guanylate cyclase activator 2a	<i>Guca2a</i>	NM_008190	Activator of guanylate cyclase	0.45
Calmodulin-like 3	<i>Calml3</i>	NM_027416	Calcium signaling	0.46
Shisa homolog 3	<i>Shisa3</i>	AV277495	FGF and WNT signaling	0.46
Histidine ammonia lyase	<i>Hal</i>	L07645	Histidine catabolism	0.46
LRRN4 C-terminal like	<i>Lrrn4cl</i>	BB783125	Unknown	0.46
Predicted gene 9746	<i>D14Ert449e</i>	BG072279	Unknown	0.48
Rac/Cdc42 guanine nucleotide exchange factor 6	<i>Arhgef6</i>	NM_152801	Rho GTPase guanine nucleotide exchange factor	0.50

decidual development and the establishment of the placenta. Probe sets for 57 transcripts exhibited a greater than or equal to twofold change in expression between *Prl8a2* null and WT tissues. Thirty-four transcripts were significantly downregulated and 23 transcripts were significantly upregulated in the *Prl8a2* null tissues ($P < 0.05$, Tables 2 and 3). The complete dataset has been deposited at the Gene Expression Omnibus (<http://www.ncbi.nlm.nih.gov/geo/>; accession number GSE60220). Pathway analyses of the transcriptome data were not informative.

Nine genes were confirmed with decreased expression in gestation day 7.5 *Prl8a2* null implantation sites (e.g. *Klk7*, *Rimklb*, *Ccl27*, *Calm4*, *Prl4a1*, *Lipg*, *Spr2h*, *Htra3*, *Arhgef6*; Table 2, Fig. 1). These include potential decidual, endothelial and trophoblast cell targets positively regulated by PRL8A2. *Rimklb* transcripts were localized to a subset of cells within the anti-mesometrial decidual compartment of the gestation day 7.5 implantation site (Fig. 2).

Six genes were confirmed with increased expression in gestation day 7.5 *Prl8a2* null implantation sites (*Derl3*,

Herpud1, *Creld2*, *Hsp90b1*, *Ddit3*, and *Hspa5*; Table 3, Fig. 3). Each of these transcripts encodes proteins that participate in the endoplasmic reticulum (ER) stress response. *Derl3*, *Hspa5*, and *Hsp90b1* transcripts were localized to the anti-mesometrial decidual compartment and were dramatically upregulated in the *Prl8a2* null mouse (Fig. 4).

Discussion

The uterine deciduum is a transitory tissue with the responsibilities of modulating hemochorial placentation. A PRL-related cytokine, PRL8A2, is expressed in a temporally- and spatially-precise pattern within the uterine deciduum during the establishment of pregnancy. PRL8A2 facilitates pregnancy-associated uterine adaptations to physiological stressors (Alam *et al.* 2007). In this report, we identified potential targets of PRL8A2 action and determined that PRL8A2 acts in a pathway that restrains activation of decidual cell ER stress.

The ER stress response is a cellular process facilitating adaptations to harmful conditions, including cellular

Table 3 List of transcripts upregulated (greater than or equal to twofold) in implantation sites of the PRL8A2 deficient mouse.

Gene name	Symbol	GenBank accession no.	Function	Ratio (null/WT)
Platelet-derived growth factor receptor-like	<i>Pdgfrl</i>	AK004179	Similarity to ligand binding domain of <i>Pdgfr</i>	11.48
Der1-like domain family, member 3	<i>Derl3</i>	AK007348	Endoplasmic reticulum stress response	6.29
Expressed sequence tag	–	AK007420	Unknown	4.65
SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member 1	<i>Smarchb1</i>	BB820473	Chromatin remodeling	3.84
Nicotinamide nucleotide transhydrogenase	<i>Nnt</i>	BB205930	Mitochondrial enzyme, production of NADPH	3.20
CDC14 cell division cycle 14 homolog B	<i>Cdc14b</i>	AK013228	Protein tyrosine phosphatase, cell cycle control	3.10
Homocysteine-inducible, endoplasmic reticulum stress-inducible, ubiquitin-like domain member 1	<i>Herpud1</i>	NM_022331	Endoplasmic reticulum stress response	3.00
EF-hand calcium binding domain 7	<i>Efcab7</i>	BC020077	Calcium binding	2.91
Expressed sequence tag	–	BB629079	Unknown	2.84
Cysteine-rich with EGF-like domains 2	<i>Creld2</i>	AK017880	Endoplasmic reticulum stress response/ calcium binding	2.75
Expressed sequence tag	–	AK007420	Unknown	2.69
Rab9 effector protein with kelch motifs	<i>Rabepk</i>	AA217054	Facilitates transport of mannose 6-phosphate receptor	2.60
Arrestin domain containing 3	<i>Arrdc3</i>	AW556597	Associated with G protein-coupled receptor signaling	2.44
γ -aminobutyric acid A receptor, subunit α 2	<i>Gabra2</i>	BB339336	GABA-A receptor, ligand-gated chloride channel	2.39
Immunoglobulin κ constant	<i>Igkc</i>	AV057155	Light chain of antibodies	2.38
Hemochromatosis	<i>Hfe</i>	AJ306425	Iron transport	2.38
Predicted gene, EG665955	<i>EG665955</i>	BF580235	Unknown	2.23
DNA segment, Chr 13, ERATO Doi 666, expressed	<i>D13Ertd666e</i>	BG070282	Unknown	2.17
DNA-damage inducible transcript 3	<i>Ddit3</i>	NM_007837	Endoplasmic reticulum stress response	2.13
Heat shock protein 5	<i>Hspa5</i>	AJ002387	Endoplasmic reticulum stress response	2.07
Uroplakin 1B	<i>Upk1b</i>	BB427704	Member of the tetraspanin family, signal transduction	2.06
Expressed sequence tag	–	BG862223	Unknown	2.04
Heat shock protein 90, β , member 1	<i>Hsp90b1</i>	NM_011631	Endoplasmic reticulum stress response	2.00

Figure 1 Validation of expression profiles of genes downregulated in *Prl8a2* null conceptus tissues. Total RNA samples from WT (+/+) and *Prl8a2* (-/-) null gestation day 7.5 implantation sites were subjected to quantitative RT-PCR (SYBR Green, $\Delta\Delta C_t$ method) with transcript specific primer sets. Reactions were performed in duplicate. 18S rRNA served as an internal control. Please note the significant downregulation genes in the *Prl8a2* null tissues. Asterisks denote significant differences between WT and *Prl8a2* null samples, $P < 0.05$.

damage, and if severe or prolonged leads to cell death (Xu *et al.* 2005, Yoshida 2007, Zhang & Kaufman 2008). Implantation of the embryo within the uterus elicits many of the hallmarks of an inflammatory response (Finn 1986, Mor *et al.* 2011). Inflammation leads to cellular injury and activation of ER stress (Zhang & Kaufman 2008). An assortment of pregnancy-related disorders, including early pregnancy loss, preeclampsia and intrauterine growth restriction, are associated with increased decidual cell ER stress responses (Lian *et al.* 2011, Liu *et al.* 2011, Loset *et al.* 2011, Gao *et al.* 2012). Pregnancy related disease occurs when the harmful inflammatory stimuli are excessive or the decidual cell adaptations are inadequate. Consequently, during the establishment of a successful pregnancy mechanisms must exist to thwart excessive or prolonged ER stress responses, which could compromise embryo survival.

The PRL family is part of a conserved decidual cell adaptation regulatory pathway. PRL and a subgroup of PRL related genes are expressed in decidual cells of the rat, mouse, and human (Orwig *et al.* 1997a, Telgmann & Gellersen 1998). PRL has a decidual-protective role in the rat and mouse. It inhibits the expression of decidual genes that interfere with the maintenance of pregnancy (Tessier *et al.* 2001, Bao *et al.* 2007). Complementary

observations are apparent in the human. PRL is produced by decidual and its production is impaired in decidual from patients with recurrent pregnancy loss (Salker *et al.* 2010, Teklenburg *et al.* 2010a,b) and correlates with failures in optimal embryo recognition (Brosens & Gellersen 2010, Weimar *et al.* 2012). PRL8A2, a PRL-related protein, is abundantly expressed in decidual of

Figure 2 *In situ* detection of *Rimkb* mRNA within implantation sites on gestation day 7.5. (A) Schematic representation of implantation sites from day 7.5 of gestation. The black box indicates the region of images shown in (B) and (C). Gestation day 7.5 implantation sites of both WT (+/+, B) and *Prl8a2* null (-/-, C) mice were subjected to *in situ* hybridization with a *Rimkb* specific antisense RNA probe. Please note the significant downregulation of *Rimkb* mRNA in the antimesometrial compartments of *Prl8a2* null tissues.

the mouse and rat, especially within anti-mesometrial decidua (Orwig *et al.* 1997a,b,c, Rasmussen *et al.* 1997). In the absence of PRL8A2, transcripts associated with ER stress are significantly upregulated in the anti-mesometrial decidua. Insights into the mechanism of PRL8A2 action are modest. PRL8A2 is a secreted heparin-binding cytokine (Rasmussen *et al.* 1996, Wang *et al.* 2000, Alam *et al.* 2008). Although PRL8A2 is structurally related to PRL, it does not bind the PRL receptor (Rasmussen *et al.* 1996). Collectively, the results suggest that the decidual-protective functions associated with PRL may extend to other members of the PRL family, including PRL8A2.

DDIT3 is a component of the ER stress response targeted by PRL8A2 and may represent a critical modulator of the integrity of decidual cells. DDIT3 is also known as CCAAT/enhancer-binding protein (C/EBP) homologous protein (CHOP) and is a negative modulator of C/EBP transcriptional regulation (Ron & Habener 1992, Tang & Lane 2000). C/EBP β is a key transcriptional mediator of the actions of progesterone on decidual cell differentiation (Bagchi *et al.* 2006, Mantena *et al.* 2006, Wang *et al.* 2010, Ramathal *et al.* 2011). Uterine stromal cells of C/EBP β null female mice fail to undergo decidualization and are unresponsive to the actions of progesterone (Bagchi *et al.* 2006, Mantena *et al.* 2006). Progesterone signaling and C/EBP β also synergize in the differentiation of primate endometrial stromal cells to decidual cells (Pohnke *et al.* 1999,

Figure 3 Validation of expression profiles of genes upregulated in *Prl8a2* null conceptus tissues. Total RNA samples from WT (+/+) and *Prl8a2* (-/-) null gestation day 7.5 implantation sites were subjected to quantitative RT-PCR (SYBR Green, $\Delta\Delta C_t$ method) with transcript specific primer sets. Reactions were performed in duplicate. 18S rRNA served as an internal control. Please note the significant upregulation genes in the *Prl8a2* null tissues. Asterisks denote significant differences between WT and *Prl8a2* null samples, $P < 0.05$.

Figure 4 *In situ* detection of *Derl3*, *Hspa5*, *Hsp90b1* mRNA within implantation sites on gestation day 7.5. (A) Schematic representation of implantation sites from day 7.5 of gestation. The black box indicates the region of images shown in (B-C). Serial cryosections from gestation day 7.5 implantation sites of both WT (+/+, B, D and F) and *Prl8a2* null (-/-, C, E and G) mice were subjected to *in situ* hybridization with a gene specific antisense RNA probes. Please note the significant upregulation of *Derl3*, *Hspa5*, and *Hsp90b1* in the anti-mesometrial compartment of the *Prl8a2* null uterus.

Christian *et al.* 2002a,b, Kannan *et al.* 2010). This leads us to speculate that by restraining DDIT3 expression, PRL8A2 effectively facilitates the actions of progesterone and C/EBP β on decidual cell development and integrity.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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