Developmental Biology 430 (2017) 325–336

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/locate/developmentalbiology

# PTHrP is essential for normal morphogenetic and functional development of the murine placenta

Chloe Duval[a,b], Mark R. Dilworth[b], Simon J. Tunster[c,1], Susan J. Kimber[a], Jocelyn D. Glazier[b,*]

[a] Division of Cell Matrix Biology and Regenerative Medicine, School of Biological Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester M13 9PT, UK

[b] Maternal and Fetal Health Research Centre, Division of Developmental Biology and Medicine, School of Medical Sciences, Faculty of Biology, Medicine and Health, University of Manchester, St Mary's Hospital, 5th Floor (Research), Oxford Road, Manchester M13 9WL, UK

[c] Cardiff School of Biosciences, Cardiff University, Cardiff, Wales CF10 3AX, UK

## 1. Introduction

Parathyroid hormone-related peptide (PTHrP) is a highly conserved and multifunctional protein that participates in a variety of complex signalling pathways (Maioli and Fortino, 2004; Philbrick et al., 1996; Wysolmerski and Stewart, 1998). The broad distribution of PTHrP across adult and fetal tissues implies that constitutive expression of PTHrP is crucial for functional regulation in multiple tissues, underpinned by the ability of PTHrP to elicit a broad range of physiological effects through its multiple pleiotropic actions (Clemens et al., 2001; Philbrick et al., 1996; Wysolmerski and Stewart, 1998). Diversity in the physiological actions of PTHrP arises from its complex translational and post-translational processing, giving rise to three mature secretory products, each serving as distinct signalling peptides that can act through different receptors (Clemens et al., 2001; Philbrick et al., 1996; Wysolmerski and Stewart, 1998). PTHrP (1–36) contains amino acid sequence homology to parathyroid hormone (PTH) and is able to activate the PTH/PTHrP receptor, whilst PTHrP (38–94) mid-fragment and PTHrP (107–139) C-terminal fragment are thought to act through distinct receptors, although these are not well characterised (Karaplis, 2001; Luparello, 2011; Philbrick et al., 1996).

PTHrP was originally identified as the humoral factor responsible for the hypercalcaemia of malignancy (Kremer et al., 2011; Philbrick et al., 1996), but it is now clear that additional to its involvement in malignancy progression (Kremer et al., 2011), PTHrP has cellular regulatory roles in the modulation of cell survival, proliferation, apoptosis and morphogenetic differentiation (Karaplis, 2001; Lanske and Kronenberg, 1998; Luparello, 2011; Martin et al., 1997; Wysolmerski, 2012). Multiplicity of PTHrP's actions to elicit these diverse cellular responses relies on its ability to act both as a paracrine and autocrine factor (Clemens et al., 2001; Philbrick et al., 1996) and evoke intracrine regulation through its lysine/arginine-rich bipartite nuclear/nucleolar targeting sequence in the mid-region (87–107) of the PTHrP peptide (Fiaschi-Taesch and Stewart, 2003; Karaplis, 2001; Luparello, 2011). This versatility enables PTHrP to act as a key developmental factor that can regulate differentiation and growth of several tissues and organs (Clemens et al., 2001; Karperien et al., 1996; Martin et al., 1997; Philbrick et al., 1996; Wysolmerski and Stewart, 1998).

The pivotal role of PTHrP as a key developmental factor is emphasized not only by its broad expression to many fetal tissues (Burton et al., 1992; Moseley et al., 1991; Philbrick et al., 1996), but also its abundant distribution in placenta, fetal membranes and amniotic fluid, in the mouse, rat and human (Bond et al., 2008; Bowden et al., 1994; Curtis et al., 1998; Emly et al., 1994; Ferguson et al., 1992; Kovacs et al., 2002, 2001b; Wlodek et al., 2000). An important role for PTHrP in fetal development is supported by observations that absence of PTHrP expression in mice, through *Pthlh* gene ablation, results in lethality (Karaplis et al., 1994; Lanske and Kronenberg, 1998), and also that expression of only a truncated form of PTHrP leads to demise in the early-postnatal period (Miao et al., 2008; Toribio et al., 2010). Further, PTHrP is expressed very early on in embryogenesis and involved in multiple embryonic differentiation processes including blastocyst differentiation (Karperien et al., 1994; Philbrick et al., 1996; van de Stolpe et al., 1993). Additionally, PTHrP acts as a potent vasodilator of the fetoplacental vasculature to maintain low resistance blood flow (Macgill et al., 1997) and plays an important role in the regulation of fetal calcium homeostasis and placental calcium transport (Bond et al., 2008; Kovacs et al., 1996; Tucci et al., 1996).

We, and others, have previously observed that *Pthlh* gene ablation in mice results in dysregulated fetal growth associated with marked skeletal and limb defects (Bond et al., 2008; Karaplis et al., 1994; Kovacs et al., 2001a; Lanske and Kronenberg, 1998). Whilst the fetal morphological phenotype and dysregulated skeletal development of the *Pthlh*-null mutant has been investigated extensively (Amizuka et al., 1996, 1994; Karaplis et al., 1994), a detailed investigation of the morphological development of the placenta is lacking. Our previous

* Corresponding author.
*E-mail address:* j.glazier@manchester.ac.uk (J.D. Glazier).
[1] Present address: Centre for Trophoblast Research, University of Cambridge, Physiology Building, Downing Street, Cambridge CB2 3EG, UK.

http://dx.doi.org/10.1016/j.ydbio.2017.08.033
Received 7 August 2017; Accepted 28 August 2017
Available online 31 August 2017
0012-1606/ © 2017 Elsevier Inc. All rights reserved.

studies have demonstrated that placental weight, as a proxy of placental growth, was altered in *Pthlh*-null mutants compared to wild-type (WT) and was associated with a reduced fetal weight and fetal:placental weight ratio, consistent with attenuation of placental nutrient transfer efficiency (Bond et al., 2008). Additionally, we demonstrated that there is an induction of PTHrP expression in trophoblast cells in early pregnancy (El-Hashash et al., 2005), and that exogenous PTHrP (1–34) stimulates the differentiation of secondary trophoblast giant cells from the polar trophectoderm *in vitro*, through altered cellular signalling associated with increased cell proliferation, altered cytoskeletal organisation, reduced apoptosis and enhanced cell survival (El-Hashash et al., 2005, 2010; El-Hashash and Kimber, 2006). Collectively, these observations are consistent with a role for PTHrP in the regulation of trophoblast cell function and placental development.

We therefore hypothesised that PTHrP regulates the development and differentiation of the murine placenta. To address how PTHrP influences placental development and function, we have conducted a multi-faceted study in *Pthlh*-null (NL) mouse mutants comparing effects associated with a lack of endogenous tissue PTHrP expression in NL mutants to WT or heterozygous (HZ) littermates. Importantly, we have examined aspects that span gestational development of the placenta with a view to providing an integrated understanding of how PTHrP affects: (i) early trophoblast cell differentiation, (ii) morphological development of the placenta, and (iii) placental functional capacity, measured as amino acid transport capacity across the intact placenta *in vivo*.

## 2. Materials and methods

### 2.1. Animals

*Pthlh* knockout mice were generated by the targeted disruption of exon IV of the *Pthlh* gene (encoding mature murine PTHrP) in embryonic stem cells as described previously (Karaplis et al., 1994), and were the kind gift of Professor H. Kronenberg (Harvard Medical School, USA). Males and females heterozygous for *Pthlh* gene deletion were mated and the day the copulation plug found was designated embryonic day 0 (E0) of gestation (term, 19 days). Animals were housed under standard 12 h light-12 h dark cycle with free access to food and water. Housing of mice, and all experimental procedures, were carried out in accordance with the UK Animals (Scientific Procedures) Act 1986 and under the authority of UK Home Office Licences 40/3385 and 40/9775. At the end of experiments, all animals were killed by Schedule 1 procedure in accordance with the above Act.

### 2.2. Genotyping

Genotyping of fetuses was performed as described previously (Bond et al., 2008) using primers specific for *Pthlh* (sense, 5′-GCTACTGCATGACAAGGGCAAGTCC-3′; antisense, 5′-GAGCCCTG CTGAACACAGTGAACAG-3′) and *neomycin resistance* (sense, 5′-GGAGAGGCTATTCGGCTATGAC-3′; antisense, 5′-CGCATTGCATC AGCCATGATGG-3′).

### 2.3. Ectoplacental cone explant culture

To investigate how the differentiation of secondary trophoblast giant cells (TGCs) is affected by a lack of endogenous PTHrP, WT, HZ and NL embryos were generated from heterozygote matings as described above. At E8.5, embryos were dissected and embryonic somatic tissue retained for genotyping. The ectoplacental cone (EPC) was carefully dissected and cultured as described previously (El-Hashash and Kimber, 2004). The isolated EPC was put into serum-free culture containing RPMI 1640 media (Lonza, UK) supplemented with 20% knockout serum replacement (Life Technologies, UK), non-essential amino acids (1%) (PAA, UK), L-glutamine (2 μM), penicillin streptomycin (1%) and β-mercaptoethanol (0.1 mM) (Sigma Aldrich, UK). The EPC explants were cultured for 72 h on fibronectin-coated plates (50 μg $ml^{-1}$) (Millipore, UK). Phase contrast images were taken of each EPC culture at 24, 48 and 72 h using an inverted Leica DM IL LED microscope (Leica, Germany) and a Leica DFC365 FX digital camera (Leica, Germany).

### 2.4. Immunocytochemistry of EPC explants and quantification of cell number

Following 72 h in culture, EPC outgrowths were fixed in 4% paraformaldehyde (PFA) overnight at 4 °C, followed by washing in Dulbecco's PBS (DPBS). Cells were permeabilized in DPBS + 0.01% Triton X-100. Non-specific binding was blocked by incubation in 10% goat serum for 1 h at RT. EPC outgrowths were incubated with primary antibodies targeting placental lactogen II (PL-II, rabbit polyclonal, 1:1000; the kind gift of Professor M Soares, University of Kansas, USA) or cleaved caspase 3 (rabbit polyclonal; Cell Signalling Technology 9661, 1:100) or non-immune IgG controls overnight at 4 °C followed by Alexa Fluor 488 secondary antibody for 1 h at RT and counter-stained with 4′,6-diamidino-2-phenylindole (DAPI) (Sigma Aldrich, UK). Immunofluorescence was observed using an inverted 1×71 Olympus microscope (Olympus, UK). Images of EPC cultures were captured by a Retiga-SRV Fast 1394 digital camera (QImaging, UK) and used to estimate cell number and nuclear area (Image J software).

### 2.5. Histology

Histology of formalin-fixed, paraffin-embedded placental tissue was performed on sections (5 μm) stained with Harris' Haematoxylin (Sigma Aldrich, UK), and then counterstained with eosin (Sigma Aldrich, UK). Tissue was observed and photographed using an Olympus BX41 upright microscope (Olympus, UK) and QICAM Fast 1364 digital camera (QImaging, UK).

### 2.6. Quantification of placental area

The areas of the junctional (JZ) and labyrinth (LZ) zones were quantified on three separate, litter-matched mid-sagittal histological-stained sections using ImageJ software.

### 2.7. Immunohistochemistry

Endogenous peroxidase activity was blocked by incubation with 1% $H_2O_2$ (Sigma Aldrich, UK) for 1 h at RT, followed by antigen retrieval (boiling in 0.01 M sodium citrate buffer pH 6 for 10 min) and blocking of non-specific binding with 5% animal serum (host species of secondary antibody; Vector Laboratories, UK). Sections (5 μm) were incubated overnight at 4 °C with goat polyclonal primary antibody (PTHrP (N-19); Santa Cruz Biotechnology sc-9680, 1:1000), cleaved caspase 3 (rabbit polyclonal; Cell Signalling Technology 9661, 1:100) or non-immune IgG as negative control, followed by incubation with appropriate biotinylated secondary antibody (Vector Laboratories, UK) for 1 h at RT. Avidin-conjugated horseradish peroxidase (VECTASTAIN elite ABC kit, Vector Laboratories, UK) was applied for 30 min and immunoreactive staining revealed by 3,3′-diaminobenzidine (Vector Laboratories, UK). Slides were imaged as described above.

### 2.8. In situ hybridization for Tpbpa mRNA

Trophoblast specific protein alpha (*Tpbpa*) was used as a gene marker of the placental junctional zone (Lescisin et al., 1988; Tunster et al., 2010). Placental tissue was collected from fetuses of each genotype at E14, 16 and 18 respectively and fixed with 4% PFA.

Download English Version:

https://daneshyari.com/en/article/5531630

Download Persian Version:

https://daneshyari.com/article/5531630

Daneshyari.com