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Immunohistochemical analysis of Heme oxygenases, Prolactin and VEGF in the mouse placenta

Zoology

KEYWORDS: Heme oxygenase; Prolactin; VEGF; Placenta

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ABSTRACT

Heme oxygenase 1 (Hmox1), the inducible form of the rate limiting enzyme in heme degradation, is required for establishment and maintenance of pregnancy. The role of Hmox2 in placentation has been ignored although high levels of its mRNA and protein have been reported in the placenta. In this study the expression of the two Hmoxs was analysed in the late gestation mouse placenta by immunohistochemistry and higher levels of Hmox2 were found in all three regions of the placenta. The expression pattern observed suggests that the two isozymes probably have different functions in the placenta. Two Hmox1 dependent factors important for angiogenesis, namely, prolactin and Vascular Endothelial Growth Factor (VEGF) along with their receptors were also analysed. The results presented in this study clearly indicate that Hmox2 and VEGF have to be studied further to understand their role in the development of the mouse embryo and placenta.

INTRODUCTION

The placenta is essential for normal growth of the foetus. Development of the placenta has been studied extensively at the molecular level (reviewed in Watson and Cross, 2005) and defects in placentation usually result in pregnancy complications in humans. Preeclampsia is a severe pregnancy complication which manifests in women with low heme oxygenase (Hmox) activity and it is thought to be a consequence of shallow placentation (reviewed in George et al., 2014). Hmox is the rate limiting enzyme in heme degradation pathway and exists as two isozymes, the inducible Hmox1 and the constitutive Hmox2 which are encoded by separate genes. While both isozymes degrade heme to equimolar amounts of CO, Fe^{2+} and biliverdin, it is Hmox1 that has been studied extensively in the last two decades since the discovery of its protective properties which is mediated by end products of its catalytic activity (reviewed in Maines and Gibbs, 2005). Interest in understanding the role of Hmoxs in pregnancy began soon after the phenotype of the Hmox1 null mutation in mouse was reported from Tonegawa's laboratory. Extensive prenatal lethality of Hmox1 knockout (KO) embryos was observed and the viable 10.5 days post coitum (dpc) embryos had very small placenta (Poss and Tonegawa, 1997; Poss 1998). Since then, studies in the Hmox1 KO mouse model reported independently by the laboratories of Stevenson and Zenclussen have deciphered the role of this isozyme in the establishment and maintenance of pregnancy (reviewed in Zenclussen et al., 2015). Development of the placenta was affected in wild type (WT) and heterozygous (HET) embryos harvested from HET timed-matings (Zhao et al., 2009). The spongiotrophoblast layer was thinner and disorganized and this was attributed to partial deficiency of Hmox1 in the HET mother. The authors concluded that insufficient development of the placenta caused the death of the Hmox1-KO embryos. It was later shown by the same group that Hmox1 is required for spiral artery remodelling as mRNA levels of several angiogenic factors were found to be lower in the decidua of HET placentas (Zhao et al., 2011). In the first report of the Hmox1 targeted KO mice (Poss and Tonegawa, 1997), Hmox1-WT and Hmox1-HET mice obtained from HET breeding pairs were in the expected Mendelian ratio whereas only 5% Hmox1-KO mice were obtained from these breeding pairs. If defective placentation due to partial Hmox1 deficiency in Hmox1-HET females was the cause of embryonic lethality, then one would expect the survival of progeny of all the three genotypes to be affected. Extensive prenatal lethality of only the Hmox1-KO embryos suggested that some factors dependent on Hmox1 expression in the embryo also contribute to its growth and survival.

Angiogenic factors whose expression was dependent on Hmox1 could be likely candidates as there is sufficient evidence for the role of Hmox1 in angiogenesis (reviewed in Loboda et al., 2008) and this process is crucial for normal development of the placenta and embryo. In the present study two factors were identified for analysis in the 16.5 dpc placenta. Prolactin (PRL) hormone and its receptor (PRLR), was chosen as placenta is an extra-pituitary site for synthesis of this hormone and PRL mediated increase in Hmox1 expression and Hmox activity was shown in human endothelial cells (Malaguarnera et al., 2002). In the same study PRL mediated *in vitro* angiogenesis was also found to be dependent on Hmox1. Vascular Endothelial Growth Factor (VEGF) and its receptors VEGFR1/Flt1 and VEGFR2/Flk1 were an obvious choice as this growth factor and its receptors are important regulators of angiogenesis (reviewed in Takahashi and Shibuya, 2005). In addition there are several studies in which VEGF expression was induced in various cell types by diverse activators of Hmox1 and the increase of VEGF was dependent on Hmox1 (reviewed in Dulak et al., 2008). As there is paucity of immunolocalization studies of PRL, VEGF and their receptors in the late gestation mouse placenta their expression was analysed in the 16.5 dpc mouse placenta. It was necessary to first determine the normal expression pattern of the Hmoxs as in 2009, Zhao et al., reported higher levels of Hmox2 mRNA and protein in 15.5 dpc Hmox1-HET placentas. The immunolocalization of the two angiogenic factors was compared to that of the Hmoxs in order to find out if there was any correlation with the expression pattern.

MATERIALS AND METHODS

Materials: Routine chemicals used were from Merck, Sigma and Qualigens. Antibodies and other reagents used for immunohistochemistry - Hmox1 and Hmox2 antibodies (Enzo Life Sciences); HRP-conjugated secondary antibodies, PRLR and VEGF antibodies (Abcam); PRL, VEGFR1/Flt1 and VEGFR2/Flk1 antibodies, goat serum and rabbit serum (Santa Cruz Biotechnology); 3, 3' diaminobenzidine (DAB) solution (Vector Laboratories) and hematoxylin stain (Sigma).

Animals: Breeding pairs of wild type FVB/C57BL/6 mice from the FVB/C57BL/6^{Hmox1} line were a kind gift from Dr. Satyajit Rath of NII, New Delhi. The colony was established and maintained at 20-24 ^{0}C, 40-70 % humidity and a 14 h light/ 10 h dark cycle. Animals were housed in an Individually Ventilated Caging System (make Citizen Industries) to maintain them in a sterile environment. Animal husbandry and use were as per CPCSEA guidelines and the experimental protocol was approved by the Institutional Animal Ethics Committee of the Department of Zoology, University of Delhi.

Harvesting of 16.5 dpc placenta: Two to six months old mice were used for experiments. Timed-matings were set in conventional cages with virgin females and proven stud males. The female was placed in the cage of the male in the evening and checked for the presence of

the plug the next morning. A female was left with the male for a maximum of 5 days after which it was discarded if no plug was detected. The day on which the plug was detected was considered as 0.5 dpc. Embryos along with their placenta were harvested at 16.5 days of gestation as follows. The pregnant female was euthanized by cervical dislocation and dissected to expose the uterus. The uterine horns were cut and placed in a petri dish containing phosphate-buffered saline A (PBSA) at room temperature (RT). The number of concepti and resorptions were noted and each conceptus was collected in a petri dish containing PBSA by cutting the uterus transversely on either side of the conceptus. The embryo along with its placenta was dissected out carefully and separated from its placenta by cutting the blood vessels. The PBSA was changed and the embryo was photographed with a digital camera (Nikon Coolpix 8400) attached to a stereozoom microscope (Nikon SMZ800 with epi-illumination). After this the weight of the embryo and placenta was noted and these were fixed in 4% paraformaldehyde at 4 ^{0}C for 21 h, then dehydrated by passing through ascending grades of alcohol from 70% to absolute alcohol and stored at -20 ^{0}C in absolute alcohol till use for immunohistochemistry. Lateral view images of the embryos were used for determining the developmental stage using Theiler staging criteria and placenta of embryos in the correct developmental stage for 16.5 days were analysed.

Immunohistochemistry: The placenta was thawed by placing in a water bath at 37 ^{0}C and then cleared by passing through ethanol/xylene (1:1) for 15 mins and xylene (two changes of 30 mins each). This was followed by embedding in paraffin wax at 62 ^{0}C (three changes of 1 h, 1.5 h and 2 h) after which it was transferred to a cavity block containing molten wax. The placenta was placed with the decidual side on the top. After the wax had solidified, the blocks were stored at 4 ^{0}C till the tissue was sectioned.

Radial sections of 5 µm thickness were cut and only the mid-radial sections were stretched by floating in a water bath at 55 ^{0}C. Stretched sections were collected on Poly-L-Lysine coated glass slides, dried in an incubator at 37 ^{0}C for 1 hour and stored at RT.

For immunohistochemical localization of the various proteins, the sections were dewaxed by passing through xylene (3 changes), and rehydrated by passing through descending grades of ethanol (from absolute to 30% ethanol) for 5 mins each and finally in deionized water. Sections were transferred to 1 mM EDTA solution and heated for 20 mins in a water bath at 90 ^{0}C for antigen retrieval. After the sections had cooled to RT these were washed in deionized water for 5 mins. Next the endogenous peroxidase was quenched by incubating the sections in 3% hydrogen peroxide (prepared in methanol) for 15 mins in the dark. The hydrogen peroxide was washed off with 0.1% PBST (four washes for 5 mins each) after which the sections were incubated with blocking buffer (5% goat serum and 1% BSA in 0.1% PBST) for 2 h at RT in a humidified chamber. Next, the blocking buffer was replaced with primary antibody at 1:100 dilution in 1.5% goat serum in 0.1% PBST and left at 4 °C for 18 h. Next day, the sections were washed twice in 0.1% PBST for 5 mins each and incubated with HRP-conjugated secondary antibody at 1:400 dilution in 1.5% goat serum in 0.1% PBST for 1 hour at RT. This was followed by three washes in 0.1% PBST for 5 mins each. Next the sections were then incubated in freshly prepared DAB solution for 1 min and then washed in deionized water for 5 mins. For immunohistochemical localization of PRL rabbit serum was used instead of goat serum. The sections were counterstained with hematoxylin stain for 30 secs and differentiated in tap water for 5 mins. After this, the sections were washed in deionized water for 5 mins, then dehydrated by passing through ascending grades of ethanol (30% to absolute ethanol) for 5 mins each, cleared in xylene (twice for 5 mins each), mounted in DPX and air dried at RT.

Imaging and quantitation: The sections were viewed at 20X magnification in a stereozoom microscope (Nikon SMZ1000) and imaged using Nikon digital camera attached to the microscope. For cellular details, the sections were imaged at 100X and 400X magnification with a digital camera (Nikon Digital Sight DS-Fil) attached to an inverted microscope (Nikon Eclipse TS100). For better representation of the immunohistochemical staining at 400X magnification, three images in each region of the placenta were taken for each section.

The protein signal was represented by brown colour localized to the antigen in the tissue section and it was quantitated using ImageJ software. First the scale was set to 50 µm with line selection tool and the scale bar. Next, the image was split into red, green and blue channels using 'RGB stack' command in the 'Image' menu command. In the green channel a defined threshold for positive staining was manually adjusted for each image using the original JPEG image of the section as a reference. The scale bar was erased in order to avoid including it in the calculation. Next 'Set measurements' was opened in the 'Analyse' menu and the parameters that were to be recorded in the result were selected. After this the integrated density values were obtained using the 'Measure' option in 'Analyze' menu and the result was saved in MS excel format. Three images were quantitated for each region of one placenta and a total of five placentas from three females were analyzed. The data was expressed as the mean integrated density of fifteen images for each region along with the standard error of mean.

Statistical analysis: SigmaPlot 12.5 software was used for statistical analysis of IHC data obtained from the junctional zone and labyrinth of five placentas. The signal intensities of seven different proteins in each region were compared as follows. First the Normality (Shapiro-Wilk Test) and Equal Variance tests were performed to determine whether the data was normally distributed. After this One Way Analysis of Variance (ANOVA) with Tukey's HSD (Honest Significant Differences) post hoc test was done to compare the mean integrated density values of the seven proteins. Statistical significance was determined at $P < 0.05$.

RESULTS AND DISCUSSION

Hmoxs: A representative result of immunohistochemical staining for the Hmoxs obtained from five placentas are presented in Figure 1. Mid-radial sections stained for Hmox1 and Hmox2 are shown at 20X magnification in Fig. 1A. The regions enclosed within rectangles in the Hmox1 panel were imaged at 100X magnification and are shown in the middle panel of Fig. 1A. The three zones of the placenta namely the decidua, the junctional zone (JZ) and the labyrinth were clearly visible. In the labyrinth region foetal blood vessels, placental villi and maternal sinuses could be identified. The 20X magnification images showed that both the Hmoxs were expressed in the three zones with the overall staining intensity for Hmox2 being higher. A differential staining pattern was observed for Hmox1 in the three regions. Maximum staining was observed in the spongiotrophoblasts of the JZ. Intermediate staining was observed in the decidua and least staining was observed in the labyrinth. In contrast, the staining for Hmox2 was uniform in all three regions of the placenta.

The 400X magnification images of the decidua, JZ and labyrinth are shown in Fig. 1B. Both the Hmoxs were present in the cytoplasm of the decidual cells and Hmox2 staining was more intense. Nuclear staining for Hmox1 was observed in most of the decidual cells whereas only a few of these cells were positive for Hmox2. In the JZ, spongiotrophoblasts, trophoblast giant cells and glycogen cells were visible. Hmox1 was present in the nucleus and cytoplasm of most of the spongiotrophoblasts and glycogen cells. In the trophoblast giant cells it was present in the cytoplasm only. Hmox2 was present in the cytoplasm of these cells. The nuclear staining for Hmox2 in the JZ did not show a consistent pattern in the five placentas. In the labyrinth region, mononuclear trophoblast cells and syncytiotrophoblasts could be identified. The former cell type lines the maternal blood sinuses whereas the latter form the outer layers of the foetal capillaries (Watson and Cross, 2005). Faint cytoplasmic staining

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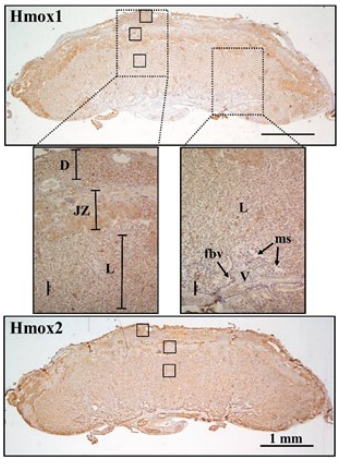
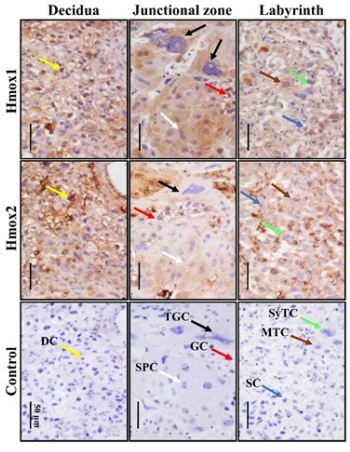


1A

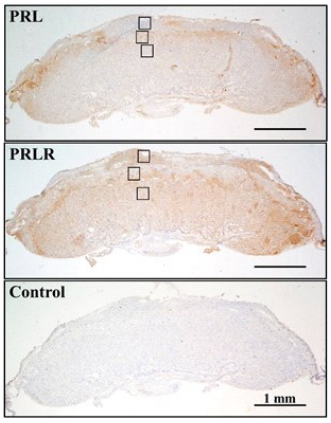


1B

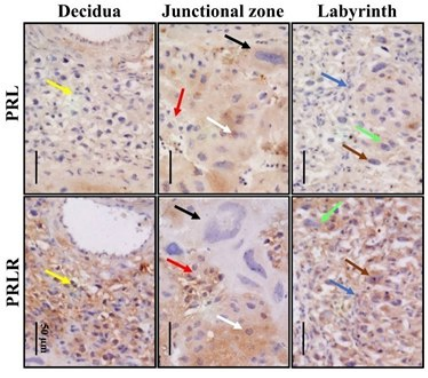
Figure 1: (A) Immunohistochemical staining of mid-radial sections of 16.5 dpc placenta with Hmox1 and Hmox2 at 20X and 100X magnifications. The rectangles and squares in the 20X images show the areas imaged in these sections at 100X and 400X magnifications respectively. The middle panel shows 100X magnification images of two regions of the Hmox1 section. (B) 400X images showing the staining in different zones of the placenta for Hmox1 and Hmox2. The 'No primary antibody' control presented in the bottom panel shows no background staining. The cell types are indicated by arrows of different colours. Yellow - Decidual cell (DC); Black - Trophoblast giant cell (TGC); White - Spongiotrophoblast cell (SPC); Red - Glycogen cell (GC); Brown - Mononuclear trophoblast cell (MTC); Green - Syncytiotrophoblast cell (SyC) and Blue - Stromal cell (SC). D - Decidua; JZ - Junctional zone; L - Labyrinth

was observed for Hmox1 in these two cell types and in most of the stromal cells whereas the staining intensity for Hmox2 was higher in all these three cell types. Strong Hmox1 and Hmox2 signals in the spongiotrophoblasts were first reported in the 16 day rat placenta (Kreiser et al., 2003). The authors however did not comment on the signal obtained in the decidua and labyrinth regions. In the following years two other groups reported high intensity staining for Hmox1 in the spongiotrophoblasts of the 14.5 dpc mouse placenta (Watanabe et al., 2004, Zhao et al., 2009). Both studies reported Hmox2 mRNA levels in mid- and late gestation placentas but presented no immunohistochemical analysis of Hmox2. The cytoplasmic staining for Hmox1 in trophoblast giant cells reported here is not consistent with the absence of staining reported in these cells at 14.5 dpc (Watanabe et al., 2004). These studies did not specify whether the staining was nuclear or cytoplasmic. Nuclear localization of Hmox1 was first reported in NIH3T3 cells (Lin et al., 2007) and translocation to the nucleus was associated with decrease in Hmox activity. The authors speculated that nuclear localization of Hmox1 probably upregulated genes that were cytoprotective. There is no report for nuclear localization of Hmox2 so far. The present study is the first report of detailed immunohistochemical analysis of the Hmoxs in the placenta.

PRL, VEGF and their receptors: The immunohistochemical staining for PRL and PRLR, VEGF and its receptors in mid-radial sections is presented in Figs. 2 and 3. Comparison of the images at 20X magnification (Figs 2A and 3A) with the corresponding Hmox images revealed similarities in the staining pattern and differences in the staining intensities. Differential staining in the three regions of the placenta similar to the Hmox1 pattern was observed for PRL and both the VEGF receptors. Staining intensity was highest in the spongiotrophoblasts of the junctional zone for the three proteins. Intermediate staining of comparable intensity was observed in the decidua and labyrinth for the two VEGF receptors whereas very little or no staining was visible for PRL in these regions. The 'No primary antibody' control for PRL (Fig. 2A) showed no background indicating that the staining was specific. For PRLR and VEGF uniform staining was observed in the three regions of the placenta similar to the Hmox2 staining pattern.



2A

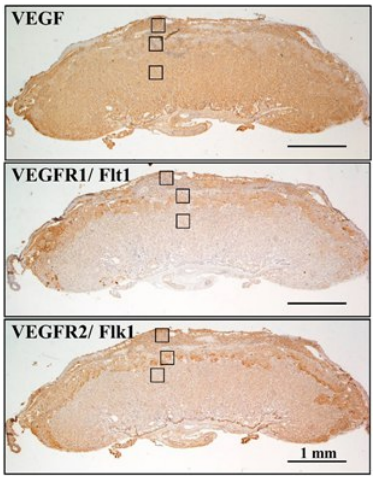


2B

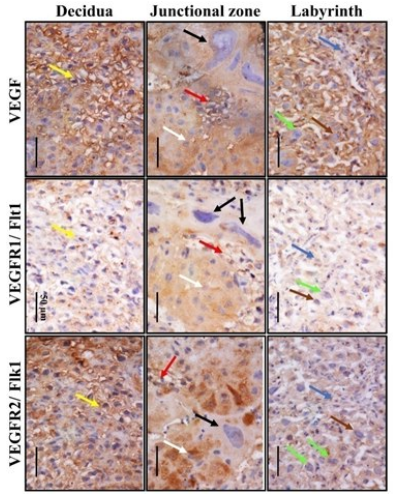
Figure 2: (A) Immunohistochemical staining of mid-radial sections of 16.5 dpc placenta with PRL and PRLR at 20X magnification. The squares in the 20X images show the areas imaged in these sections at 400X magnification. The 'No primary antibody' control presented in the bottom panel shows no background staining. (B) 400X images showing the staining in different zones of the placenta for PRL and PRLR. The cell types are indicated by arrows of different colours and details are mentioned in the legend of Fig. 1.

The 400X magnification images (Figs. 2B and 3B) showed that all five proteins were present in the cytoplasm of the decidual cells. Least staining was observed for PRL and VEGFR1/Flt1. Nuclear staining was also observed for PRLR, VEGF and VEGFR2/Flk1, a pattern similar to Hmox1. None of the decidual cells showed nuclear staining

for PRL. Very few decidual cells showed nuclear staining for VEGFR1/Flt1, a pattern similar to Hmox2. In the JZ all the five proteins were present in the cytoplasm of the spongiotrophoblasts, glycogen cells and trophoblast giant cells. Nuclear staining was observed for these proteins in many of the spongiotrophoblasts and glycogen cells, a similarity with the Hmox1 pattern. In the labyrinth, PRL was absent in the syncytiotrophoblasts and stromal cells. Cytoplasmic staining for the remaining four proteins was observed in these cells with the intensity for



3A



3B

Figure 3: (A) Immunohistochemical staining of mid-radial sections of 16.5 dpc placenta with VEGF, VEGFR1/Flt1 and VEGFR2/Flk1 at 20X magnification. The squares in the 20X images show the areas imaged in these sections at 400X magnification. (B) 400X images showing the staining in different zones of the placenta for VEGF, VEGFR1/Flt1 and VEGFR2/Flk1. The cell types are indicated by arrows of different colours and details are mentioned in the legend of Fig. 1.

VEGF being the highest. Mononuclear trophoblasts were positive for all the five proteins. Nuclear staining was observed in the three cell types of the labyrinth mainly for VEGFR1/Flt1.

Presence of PRL protein in the mid- and late gestation mouse placenta has been shown by immunoblotting (Harigaya et al., 1997). However there are no reports of immunohistochemical analysis of PRL and PRLR in the mouse placenta. VEGF expression has been analysed in the 17 d mouse placenta by in situ hybridization (Breier et al., 1992) and VEGF transcripts were found to be abundant in the labyrinth. The immunohistochemistry results presented here provide evidence for the presence of VEGF proteins in all the cell types of the labyrinth. VEGF receptor expression in the developing mouse embryo and placenta was also reported a few years later (Breier et al., 1995). VEGFR1/Flt1 transcript signals were high in the spongiotrophoblasts of the 14.5 dpc placenta and low in the labyrinth region while the VEGFR2/Flk1 transcript signals were detected mainly in the labyrinth region. The immunohistochemical analysis for the two receptors presented in this study show a similar pattern of differential staining for the two VEGF receptors in the 16.5 dpc placenta. The significance of this difference is not clear and would require further analysis.

Semi-quantitative analysis of immunohistochemistry results: In order to compare the protein signals in each region of the placenta, the mean integrated density for each protein determined using ImageJ software was plotted as a clustered bar graph and is presented in Figure 4. Hmox2 signal was higher than Hmox1 in both the JZ and labyrinth. Signals for PRLR, VEGF and its receptors were also of high intensity in both regions of the placenta.

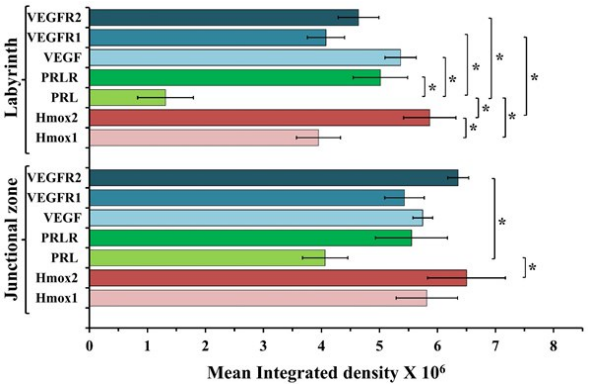


Figure 4: Immunohistochemical signals for the analysed proteins in the JZ and labyrinth are plotted as Mean integrated density x 10^6 ± SEM (n=5). An asterisk indicates significant difference between two groups as determined by Tukey's HSD post-hoc test. Statistical significance was determined at $P < 0.05$.

Statistical analysis revealed that in the JZ significant differences were observed only between Hmox2-PRL and PRL-VEGFR2/Flk1 proteins. In the labyrinth region, PRL signal was very less and significantly different from all the six proteins. In addition significant differences were observed between Hmox1-Hmox2 and Hmox2-VEGFR1/Flt1. Data for the decidua did not pass the Normality test and therefore One Way ANOVA could not be performed.

This semi-quantitative analysis only gives an idea of the protein signals relative to each other in the JZ and labyrinth region of the 16.5 dpc mouse placenta and therefore cannot be compared to reports where protein levels were determined by immunoblotting.

CONCLUSIONS

Hmox1 is expressed differentially in the three regions of the 16.5 dpc mouse placenta with a maximum in the spongiotrophoblasts of the JZ, intermediate expression in the decidua and least in the labyrinth region. PRL and the two VEGF receptors also showed such a differential expression pattern. The differential expression suggests a regulatory role for these proteins. Hmox2 expression was uniform in the three regions of the placenta and was higher than Hmox1. PRLR and VEGF expression pattern was similar to that of Hmox2. The uniform expression pattern suggests a constitutive role for these in the placenta.

Examination of the cellular expression at 400X magnification revealed that the decidual cells expressed all the seven proteins in the cytoplasm. This is not unexpected as decidua comprises the maternal component of the placenta. The novel observations in the JZ and labyrinth region was that both Hmoxs appeared to be co-

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expressed in the various cell types but the expression is higher for Hmox2 in the labyrinth region. This and the nuclear expression of Hmox1 suggest that the two Hmoxs perform different functions in the placenta. Among the two proteins identified as likely candidates for Hmox1 dependent factors important for survival of the embryos, it appears that VEGF may be more important than PRL as the latter is not expressed in most of the cells in the labyrinth region. Further studies in the Hmox1-KO embryos and placentas would provide more insight into the relative importance of the two Hmoxs and VEGF during mouse embryogenesis.

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REFERENCES

1. Breier, G., Albrecht, U., Sterrer, S., & Risau, W. (1992). Expression of vascular endothelial growth factor during embryonic angiogenesis and endothelial cell differentiation. Development, 114, 521-532.
2. Breier, G., Clauss, M., & Risau, W. (1995). Coordinate Expression of Vascular Endothelial Growth Factor Receptor - 1 (flt-1) and Its Ligand Suggests a Paracrine Regulation of Murine Vascular Development. Dev Dyn, 204, 228-239.
3. Dulak, J., Deshane, J., Jozkowicz, A., & Agarwal, A. (2008). Heme Oxygenase-1 and Carbon Monoxide in Vascular Pathobiology: Focus on Angiogenesis. Circulation, 117, 231-241.
4. George, E. M., Warrington, J. P., Spradley, F. T., Palei, A. C., & Granger, J. P. (2014). The heme oxygenases: important regulators of pregnancy and preeclampsia. Am J Physiol Regul Integr Comp Physiol, 307, R769-R777.
5. Harigaya, T., Komori, M., Kawakami, T., Watanabe, H., & Abe, M. (1997). Expression of Prolactin Gene in Mouse Placenta During Late Pregnancy: Detection of mRNA and Its Translation Product. Endocr J, 44, 155-161.
6. Kreiser, D., Kelly, D. K., Seidman, D. S., Stevenson, D. K., Baum, M., & Dennery, P. A. (2003). Gestational Pattern of Heme Oxygenase Expression in the Rat. Pediatr Res, 54, 172-178.
7. Lin, Q., Weis, S., Yang, G., Weng, Y. H., Helston, R., Rish, K., . . . Dennery, P. A. (2007). Heme Oxygenase-1 Protein Localizes to the Nucleus and Activates Transcription Factors Important in Oxidative Stress. J Biol Chem, 282, 20621-20633.
8. Loboda, A., Jazwa, A., Grochot-Przeczek, A., Rutkowski, A. J., Cisowski, J., Agarwal, A., . . . Dulak, J. (2008). Heme Oxygenase-1 and the Vascular Bed: From Molecular Mechanisms to Therapeutic Opportunities. Antioxid Redox Signal, 10, 1767-1812.
9. Maines, M. D., & Gibbs, P. E. (2005). 30 some years of heme oxygenase: from a "molecular wrecking ball" to a "mesmerizing" trigger of cellular events. Biochem Biophys Res Commun, 338, 568-577.
10. Malaguarnera, L., Pilastro, M. R., Quan, S., Ghattas, M. H., Yang, L., Mezentsev, A. V., . . . Kappas, A. (2002). Significance of heme oxygenase in prolactin-mediated cell proliferation and angiogenesis in human endothelial cells. Int J Mol Med, 10, 433-440.
11. Poss, K. D. (1998). Examination of Diverse Physiological Functions of Mammalian Heme Oxygenases by Gene Targeting. Ph.D. thesis, Department of Biology, Massachusetts Institute of Technology.
12. Poss, K. D., & Tonegawa, S. (1997). Heme oxygenase 1 is required for mammalian iron reutilization. Proc Natl Acad Sci U S A, 94, 10919-10924.
13. Takahashi, H., & Shibuya, M. (2005). The vascular endothelial growth factor (VEGF)/ VEGF receptor system and its role under physiological and pathological conditions. Clin Sci (Lond), 109, 227-241.
14. Watanabe, S., Akagi, R., Mori, M., Tsuchiya, T., & Sassa, S. (2004). Marked Developmental Changes in Heme Oxygenase-1 (HO-1) Expression in the Mouse Placenta: Correlation Between HO-1 Expression and Placental Development. Placenta, 25, 387-395.
15. Watson, E. D., & Cross, J. C. (2005). Development of Structures and Transport Functions in the Mouse Placenta. Physiology, 20, 180-193.
16. Zenclussen, M. L., Linzke, N., Schumacher, A., Fest, S., Meyer, N., Casalis, P. A., & Zenclussen, A. C. (2014). Heme oxygenase-1 is critically involved in placentation, spiral artery remodeling, and blood pressure regulation during murine pregnancy. Front Pharmacol, 5, 291.
17. Zhao, H., Azuma, J., Kalish, F., Wong, R. J., & Stevenson, D. K. (2011). Maternal Heme Oxygenase 1 Regulates Placental Vasculature Development via Angiogenic Factors in Mice. Biol Reprod, 85, 1005-1012.
18. Zhao, H., Wong, R. J., Kalish, F. S., Nayak, N. R., & Stevenson, D. K. (2009). Effect of Heme Oxygenase-1 Deficiency on Placental Development. Placenta, 30, 861-868.