MRI of Placental Angiogenesis in 'Tetraploid Embryo Rescue' of PKBalpha/Akt1- deficient Mice Using biotin-BSA-GdDTPA Enhanced 3D-MRI

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Introduction:

Angiogenesis plays a vital role in placental development. PKB/Akt regulates many cellular and physiological processes through phosphorylation of numerous substrates, affecting glucose metabolism, transcription, cell cycle regulation, survival, inflammation and angiogenesis [1]. In a recent study, mouse embryos (E14.5) deficient in PKBalpha (PKB isoform, widely expressed in mouse placenta and the embryo proper) were shown to be smaller with disordered fetal vasculature and increased neonatal mortality [2]. Placentas of PKBalpha null fetuses were smaller as compared to wildtype, suggesting a novel role for PKBalpha in placental and fetal development. Our study aimed at differentiating between the role of placental PKBalpha versus the role of fetal PKBalpha in pregnancy progression and overall fetal wellbeing and development. Macromolecular contrast enhanced MRI was applied for the assessment of normal and impaired placental function, specifically focusing on 'tetraploid embryo rescue': To bypass fetal damage which is subsequent to placental insufficiency, the PKBα null embryo is provided with a healthy placenta.

Materials and Methods:

Animals: Pregnant ICR females (E18.5) bearing PKBalpha+/+, +/-, -/- fetuses (C57Bl/6 background, i.e. B6) with either native or tetraploid- GFP placentas.

Tetraploid rescue: For the tetraploid placentas, superovulated ICR females were mated with B5/EGFP males to produce GFP positive placentas for easier ex vivo detection. The pregnant females were sacrificed on E1.5 to collect late two cell embryos, by flushing the oviduct. Following an electric pulse, embryos were incubated for 30-40 minutes during which fusion took place and created 1 cell tetraploid embryos that were then cultured overnight. The embryos for rescue were washed on E3.5 from five weeks old B6/PKBalpha +/- females that were superovulated and mated with B6/PKBalpha -/- males. Immunosurgery was performed by lysis of trophectoderm cells to isolate inner cell mass (ICM) from the E3.5 blastocysts. Aggregation of 2 tetraploid embryos sandwiched around a single isolated ICM was performed and after 24h of incubation, aggregated blastocysts were transferred to the uterus of E2.5 pseudopregnant ICR female.

MRI experiments: Animals were placed in a 4.7T horizontal Bruker (Germany) Biospec spectrometer and a whole body excitation coil was used. Contrast agent: biotin-BSA-GdDTPA was prepared as reported [3]. After MRI, mice were injected with BSA-ROX 5min (to visualize placental vasculature ex- vivo). Placentas and fetuses were retrieved and placentas were examined for the presence of GFP.

3D-GE: A series of images with 15, 5, 30, 50, 70 degrees flip angles were acquired to determine the precontrast R1 (TR 10ms, TE 3.6ms, 2 averages, spectral width 50000Hz, matrix 128x128x64 (zero filled to 128), FOV 5x5x5 cm). Dynamic post contrast images were acquired with a 15 degrees flip angle and animals were followed for 30min after intravenous administration of biotin-BSA-GdDTPA via the tail vein.

MRI data analysis: Mean biotin-BSA-GdDTPA concentrations were derived from the precontrast R1 and using pre- and postcontrast 3D-GE-mean signal intensities at ROIs of placentas, and were used for derivation of fBV (blood volume fraction) [4].

Results:

Using biotin-BSA-GdDTPA enhanced MRI, PKBalpha -/- (null) placentas were found to have reduced blood volume as compared to PKBalpha +/+ (wildtype) when examined on E18.5. PKBalpha -/- fetuses were also reduced in size as compared to wildtype and exhibited significantly higher fetal mortality in utero. Tetraploid placentas (GFP positive, PKBalpha +/+) that exhibited normal blood volume, did not "rescue" the size of PKBalpha -/- fetuses which remained significantly smaller than wildtype. However, while the number of non- rescued PKBα -/- fetuses was found to be significantly reduced, PKBalpha -/- fetuses with tetraploid placentas were rescued from fetal death in utero, as exhibited on E18.5, just prior to parturition.

Discussion:

We have established an MRI methodology for the analysis of placental vascular function, focusing on reproductive assisted technologies as 'tetraploid embryo rescue'. Contrast enhanced MRI using biotin-BSA-GdDTPA allowed quantification of placental function by measurement of blood volume. As such, MRI allows the differentiation of vascularized resorped placentas with reduced blood volume from normal placentas. This may be useful to determine fetal death in utero prior to full resorption (data not shown). MRI was also used to assess tetraploid rescue of PKBalpha null embryos from fetal death associated with placental insufficiency. Although the tetraploid placentas did not "rescue" fetal size, they prevented PKBalpha null embryos from fetal death. Therefore, tetraploid rescue allowed us to differentiate between the roles of placental versus fetal PKBalpha in embryonic development: While placental PKBα regulates fetal survival in utero, fetal PKBα regulates fetal growth.

Figure 1.

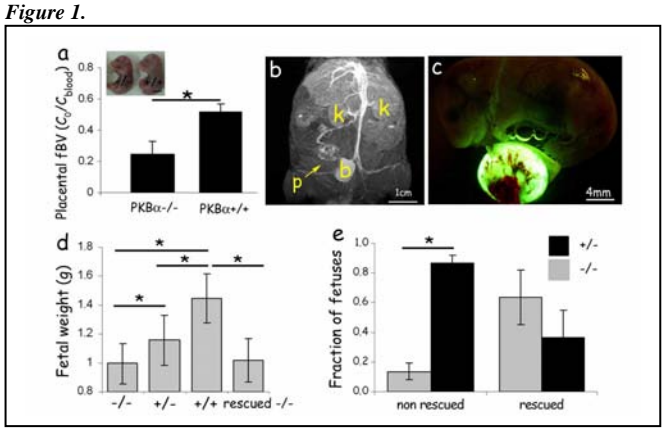

Figure 1. ***(a)*** PKBalpha -/- placentas exhibited significantly reduced blood volume (fBV) as compare to PKBα +/+, wildtype placentas on E18.5. PKBα -/- fetuses are also reduced in size as compared to wildtype (insert). Maximal intensity projection of biotin-BSA-GdDTPA enhanced tetraplod placenta ***(b)*** of a rescued E18.5 fetus (GFP-positive placenta in green and BSA-ROX-enhanced vessels in red) ***(c)***. Although the rescued PKBalpha -/- fetuses remained significantly smaller than wildtype fetuses, despite the tetraploid placentas ***(d)***, they were rescued from fetal death in utero ***(e)***.b- bladder, k- kidney, p- placenta.

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V. Plaks and E. Berkovitz contributed equally to this work

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