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Marsupial tammar wallaby delivers milk bioactives to altricial pouch young to support lung development

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ABSTRACT

Our research is exploiting the marsupial as a model to understand the signals required for lung development. Marsupials have a unique reproductive strategy, the mother gives birth to altricial neonate with an immature lung and the changes in milk composition during lactation in marsupials appears to provide bioactives that can regulate diverse aspects of lung development, including branching morphogenesis, cell proliferation and cell differentiation. These effects are seen with milk collected between 25 and 100 days postpartum. To better understand the temporal effects of milk composition on postnatal lung development we used a cross-fostering technique to restrict the tammar pouch young to milk composition not extending beyond day 25 for 45 days of its early postnatal life. These particular time points were selected as our previous study showed that milk protein collected prior to ~day 25 had no developmental effect on mouse embryonic lungs in culture. The comparative analysis of the foster group and control young at day 45 postpartum demonstrated that foster pouch young had significantly reduced lung size. The lungs in fostered young were comprised of large intermediate tissue, had a reduced size of airway lumen and a higher percentage of parenchymal tissue. In addition, expression of marker genes for lung development (BMP4, WNT11, AQP-4, HOPX and SPB) were significantly reduced in lungs from fostered young. Further, to identify the potential bioactive expressed by mammary gland that may have developmental effect on pouch young lungs, we performed proteomics analysis on tammar milk through mass-spectrometry and listed the potential bioactives (PDGF, IGFBP5, IGFBPL1 and EGFL6) secreted in milk that may be involved in regulating pouch young lung development. The data suggest that postnatal lung development in the tammar young is most likely regulated by maternal signalling factors supplied through milk.

1. Introduction

In mammals the lung has evolved as a respiratory organ to exchange gases immediately after birth (Philip, 1977). In eutherians the majority of lung development occurs during intrauterine life. The lungs of new born are at an alveolar stage of development and the key changes that occur during early postnatal life include increased alveolar number and maturation of microvasculature (Burri, 2006). Respiratory complications are frequently seen in premature infants, largely due to the incomplete development of lung (Burri, 1984). In contrast to eutherians, the marsupial young is born at an altricial stage after a short gestation and the major development occurs postnatally (Nicholas et al., 1997; Renfree, 2006; Tyndale-Biscoe and Renfree, 1988). New born marsupials are similar in development to a late eutherian foetus and the immature lung is required to develop rapidly to become fully functional. Studies in marsupial species including the Julia Creek dunnart (*Sminthopsis douglasi*) (Frappell and Mortola, 2000), tammar wallaby (*Macropus eugenii*) and gray short tailed opossum (*Monodelphis domestica*) (Szdzuy et al., 2008) have shown the lungs of newborns are comprised of a small number of large air sacs providing insignificant surface area for respiration, and the lungs are considered as functionally immature (Mess and Ferner, 2010). Therefore these marsupial newborn have adopted a unique respiratory mechanism to perform partial gaseous exchange through skin during early postnatal development to meet their oxygen demand (Frappell and Mortola, 2000; Mortola et al., 1999).

In all mammals, the extent of development at birth depends on the period of intrauterine development as the factors responsible for fetal development are provided by the maternal placenta and amniotic fluid (Ferner et al., 2009; Fowden, 1995; Fowden and Forhead, 2009; Sibley et al., 1997). Subsequently the eutherian neonate has mature organs for normal function. In contrast, development of most organs at birth in marsupials is significantly less advanced (Setiati, 1986; Szdzuy

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et al., 2008; Wilkes and Janssens, 1986). From an evolutionary perspective, the survival of these primitive mammalian progeny would have been at greater risk of extinction, but these mammals have adapted a unique lactation system to support postnatal development of their progeny (Daniel, 1993; Goldman, 2002). This development appears to rely on the factors provided through milk (Nicholas et al., 1997). Therefore the marsupial provides an alternative and unique model to understand the process of lung development and also to investigate the potential significance of milk bioactives for signalling this development. Recently we attempted to address the hypothesis of milk regulating postnatal lung maturation by culturing mouse embryonic lungs with tammar milk whey collected early in lactation (Modepalli et al., 2015). The results showed branching morphogenesis and growth of lung explants cultured with milk from day 20 lactation whey was restricted. In contrast, when an embryonic lung was cultured with milk collected between day 40 and 100 extensive branching morphogenesis and tissue growth was observed, and this effect was significantly reduced with milk collected after day 100 of lactation (Modepalli et al., 2015). This suggested that tammar milk collected prior to day 20–25 lacked the factors necessary for programming growth and development of lung, and indeed may have included factors that halt lung progression, indicating that the marsupial milk may contain both positive and negative factors to regulate lung maturation.

In the present study we have exploited the tammar wallaby as a model to better understand the potential role of milk in postnatal lung development of pouch young. To address this aim we have adopted a cross-fostering technique of transferring the pouch young at day 25 of age to a series of mothers at day 15 of lactation so that the young only receive milk from day 15 to 25 of lactation for a period of 20 days. The lungs were then analysed to determine if development was reduced. This regime therefore examined the hypothesis of temporal effects of milk composition on lung development.

2. Results

2.1. Cross-fostering the PYs to control the milk composition

The day 45 old PY were collected from both foster and control group mothers and individual PY weight and head length were measured and compared between both groups. The fostered PY were significantly reduced in head length (*P* value 0.051228) (Fig. 1A) and weight (*P* value 0.000712) (Fig. 1B) compare to the control PY.

2.2. Lung morphology of PY in foster and control groups

The size of the left lobe of the lung was assessed and morphological analyses was performed on the right lobe collected from each PY. The lungs were sectioned and stained to assess morphological development and the images were analysed using Image J software. The percentage of parenchymal tissue in lung from fostered PY was significantly higher and the percentage of respiratory lumen area was significantly lower (Fig. 2).

2.3. Developmental marker gene expression in lungs from control and foster pouch young

Comparative analysis of expression of genes for developmental markers related to postnatal lung maturation (alveolization and branching morphogenesis) was assessed by RT-PCR analysis (Fig. 3). Results showed that marker genes related to branching morphogenesis (BMP4 and WNT11), alveolization or Type-I epithelial (HOPX), Type-II epithelial (SPB), terminal and airway epithelia (AQP4) showed a significant reduction in lungs from fostered PY compared to lungs from control PY.

2.4. Milk composition - total carbohydrates, lipids and protein

The milk samples collected at the time the PY were sacrificed from both groups were analysed for concentration of total carbohydrates, lipids and protein (Fig. 4). There was no difference in the concentration of total carbohydrate and lipid in milk provided to the PY from both foster and control PY. In contrast, the total protein concentration was significantly lower in the fostered group milk samples.

2.5. Identifying secreted milk proteins during early lactation and analysed through functional categorisation

Mass spectrometry was used to identify the secreted milk proteins in milk at day 20, day 60 and day 120 lactation. Using data analysis from LC-MS/MS we identify 189, 200 and 152 proteins in milk collected from day 20, day 60 and day 120 respectively. Among these identified proteins, 93 were reported all three time points. In day 20 milk samples 55 proteins from a total of 189 were uniquely expressed only in day 20 milk sample and 28 were expressed only in milk at day 20 and day 60. Analysis of the 200 proteins reported in day 60 milk samples, 52

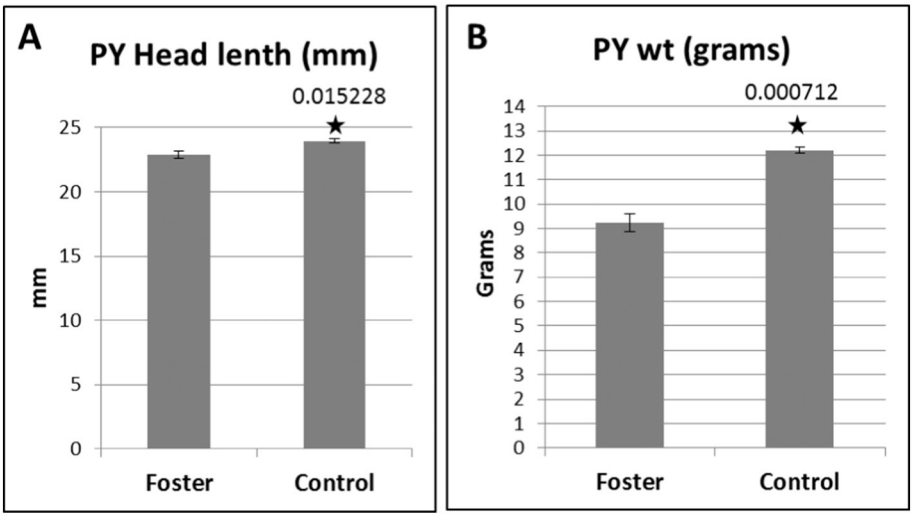

Fig. 1. The (A) head length and (B) body weight of foster and control pouch young. Data shown are mean ± SEM for 3 PY from each group. Statistically significant *P* values (<0.05) are shown with an asterisk.