Expression of aldehyde dehydrogenase family 1, member A3 in glycogen trophoblast cells of the murine placenta

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The mouse placenta is organized into three layers; a maternal layer of transformed uterine tissue called the decidua, an intermediate trophoblast layer called the junctional zone (JZ), and the innermost labyrinth layer, which contains the intertwined fetal and maternal circulations. The JZ, which has both structural and endocrine roles, includes several trophoblast subtypes including glycogen trophoblast (GlyT), spongiotrophoblast and several unique trophoblast giant cell (TGC) subtypes; spiral artery-associated TGCs, canal-associated TGCs and channel-associated TGCs. The labyrinth contains distinct trophoblast subtypes involved in fetal-maternal exchange, including two layers of transporting syncytiotrophoblast cells and a mononuclear TGC cell subtype. Beginning at embryonic day 7.5, two early placental structures, the ectoplacental cone (EPC) and the chorion contain distinct intermediary groups of progenitors that give rise to trophoblast of the JZ and labyrinth respectively.

Retinoic acid (RA) signaling regulates the differentiation of trophoblast cells in both the JZ and the labyrinth, with the source of this RA thought to be maternal decidual cells (Rossant & Cross, 2001; Yan *et al.*, 2001). In this study we analyzed by *in situ* hybridisation the placental expression of aldehyde dehydrogenase family 1, member A3 (*Aldh1a3*), an enzyme in the RA synthesis pathway (Marchitti *et al.*, 2008; Kumar *et al.*, 2012). We report *Aldh1a3* mRNA is expressed in a subset of EPC progenitor cells and is later tightly restricted to GlyT cells of the mature placenta, suggesting GlyT are a local source of RA well positioned to influence differentiation of both labyrinth and JZ trophoblast. In addition, *Aldh1a3* appears to be a new and specific marker of GlyT cells and their precursors; a useful tool for further studies into this poorly described cell type.

To further explore potential sites of RA action within the placenta we analyzed expression patterns of several other RA metabolising (*Aldh1a1, Aldh1a2*) and receptor (*Rara/b/g, Rxra/b/g, Pparg/d*) genes. *In situ* hybridization results revealed that all trophoblast progenitors and differentiated placental cell types express combinations of RA receptors that would equip them to respond to local RA. However, based on our observations, and information gained from retinoid X receptor (*Rxra* and *Rxrb*) (Wendling *et al.*, 1999) and peroxisome proliferator activated receptor (*Ppard* and *Pparg*) knock out mice (Barak *et al.*, 2002; Barak *et al.*, 1999), the majority of trophoblast cells are most likely responding through the non-classical *9-cis* RA pathway *via* RXRs dimerized with one of their many binding partners (extensively PPARs, but including RXR homodimers, vitamin D receptors, liver X receptors, and thyroid receptors). We sought to confirm this notion using RARE-LacZ reporter mice, which localises the presence of classical RA signaling through RARs. While we saw robust LacZ expression in the developing embryo, we detected minimal LacZ transgene activity in placental sections from several gestational time points, confirming that classical RA signaling appears to play a minor role in placental development.

To address whether the differentiation of trophoblast progenitor cells are influenced by endogenous RA production, we examined the effects of aldehyde dehydrogenase family 1 (ALDH1) inhibitors on trophoblast stem cell differentiation. Trophoblast stem cells were differentiated in the presence of ALDH1 inhibitors DEAB or citral, and expression of specific labyrinth (*Syna, Ctsq*) and junctional zone (*Tpbpa, Prl7b1, Prl7a2*) marker genes were analyzed by qRT-PCR. Interestingly, we found that expression of JZ cell type markers decreased as a result of reduced ALDH1 activity, suggesting differentiation or expansion of JZ cell types may be influenced by the presence of locally produced RA; *in vivo* produced by EPC progenitors and subsequently GlyT of the JZ.

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