Placenta 42 (2016) 74–83

Contents lists available at ScienceDirect

Placenta

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

Differential patterning of genes involved in serotonin metabolism and transport in extra-embryonic tissues of the mouse

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ARTICLE INFO

Article history:
Received 14 December 2015
Received in revised form
10 March 2016
Accepted 26 March 2016

Keywords:
Serotonin
Placenta
Syncytiotrophoblast
Sinusoidal trophoblast giant cells
Yolk sac

ABSTRACT

Introduction: Serotonin (5-HT) is an important neuromodulator, but recently has been shown to be involved in neurodevelopment. Although previous studies have demonstrated that the placenta is a major source of forebrain 5-HT during early forebrain development, the processes of how 5-HT production, metabolism, and transport from placenta to fetus are regulated are unknown. As an initial step in determining the mechanisms involved, we investigated the expression patterns of genes critical for 5-HT system function in mouse extraembryonic tissues.
Methods: Mid-through late gestation expression of 5-HT system-related enzymes, *Tph1*, *Ddc*, *Maoa*, and 5-HT transporters, *Sert/Slc6a4*, *Oct3/Slc22a3*, *Vmat2/Slc18a2*, and 5-HT in placenta and yolk sac were examined, with cell type-specific resolution, using multiplex fluorescent in situ hybridization to co-localize transcripts and immunocytochemistry to co-localize the corresponding proteins and neurotransmitter.
Results: Tph1 and *Ddc* are found in the syncytiotrophoblast I (SynT-I) and sinusoidal trophoblast giant cells (S-TGC), whereas *Maoa* is expressed in SynT-I, syncytiotrophoblast II (SynT-II) and S-TGC. *Oct3* expression is observed in the SynT-II only, while *Vmat2* is mainly expressed in S-TGC. Surprisingly, there were comparatively high expression of *Tph1*, *Ddc*, and *Maoa* in the yolk sac visceral endoderm.
Discussion: In addition to trophoblast cells, visceral endoderm cells in the yolk sac may contribute to fetal 5-HT production. The findings raise the possibility of a more complex regulation of 5-HT access to the fetus through the differential roles of trophoblasts that surround maternal and fetal blood space and of yolk sac endoderm prior to normal degeneration.

1. Introduction

Maternal-fetal interactions have long-term health consequences for the offspring [1,2], including neurodevelopmental disorders [3,4]. The placenta, central to the maternal-fetal interface, plays an important role in regulating these interactions. Recently we demonstrated that the placenta is a major site of fetal serotonin (5-hydroxytryptamine, 5-HT) production in mice and humans [5], providing the monoamine to the forebrain during early development. Although 5-HT is best known as a monoamine neurotransmitter in the adult brain, it was first discovered as intestine contractor and vasoconstrictor [6]. And 5-HT has been shown to modulate neurogenesis, axon guidance and axon pathway refinement during fetal brain development [7–9]. 5-HT is derived from tryptophan, an essential amino acid, first by tryptophan hydroxylase (TPH1 or TPH2) that converts tryptophan to 5-hydroxytryptophan (5-HTP) in a tetrahydrobiopterin (BH4)-dependent manner. This is followed by pyridoxal phosphate (PLP)-depenendent decarboxylation of 5-HTP to 5-HT via dopa decarboxylase (DDC) Fig. 1D; [6,10–13]. Serotonergic circuitry in the central nervous system originates early in embryonic development from a small number of brainstem neurons expressing both TPH2 and DDC. These neurons develop an extensive axonal network that innervate structures throughout the entire central nervous system [14]. Studies from our laboratory demonstrated that deregulation of fetal 5-HT signaling before serotonergic axons reach forebrain results in altered brain circuitry [7]. We also reported that at early

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http://dx.doi.org/10.1016/j.placenta.2016.03.013
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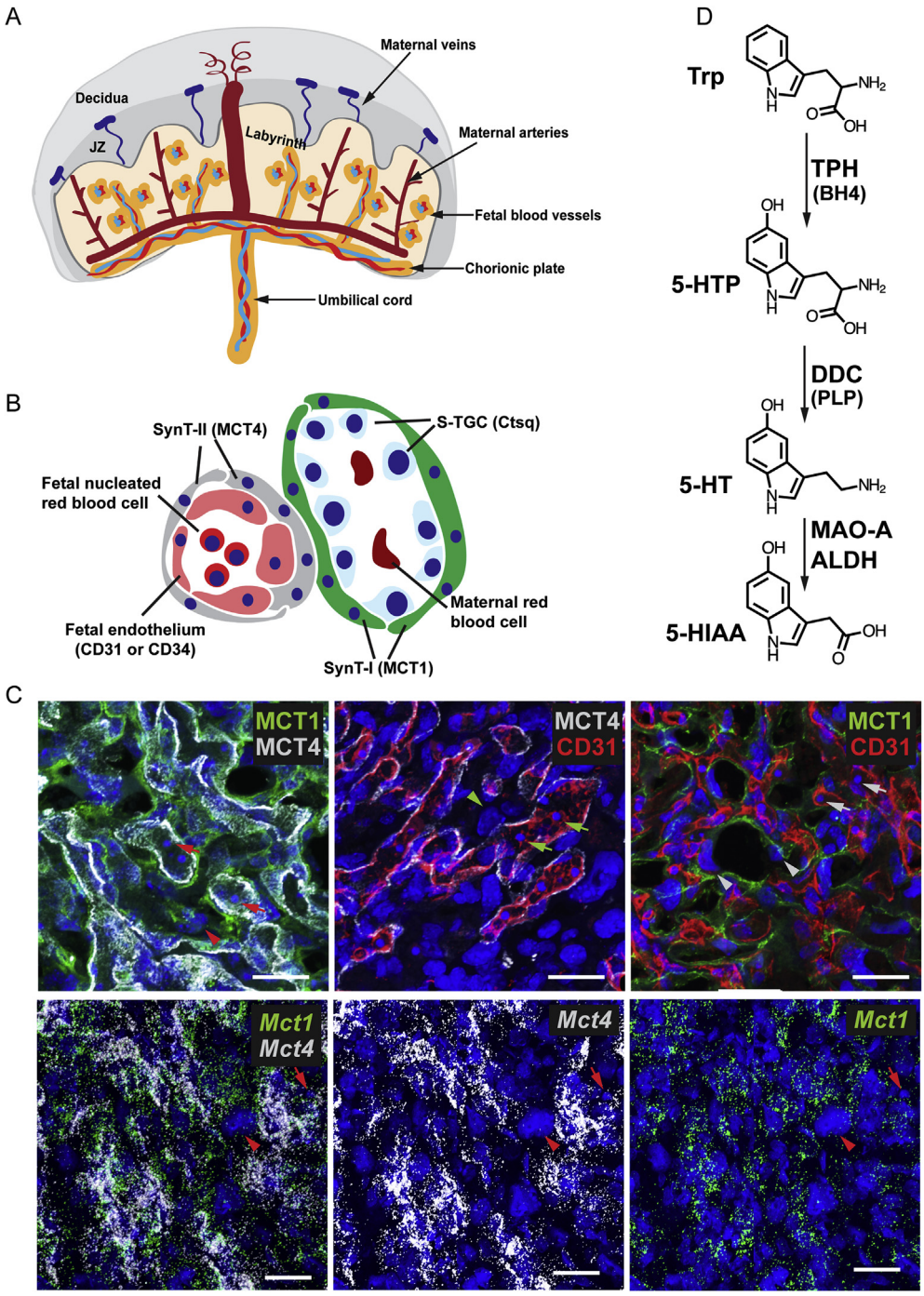

Fig. 1. Different cell types and their molecular markers in the labyrinth. (A) Diagram showing the general structure of the murine placenta. (B) Schematic drawing depicting the cell types and cell organizations in the labyrinth. Molecular markers used to label specific cell type are shown in parentheses. SynT-I, syncytiotrophoblast I; SynT-II, syncytiotrophoblast II; S-TGC, sinusoidal trophoblast giant cell. (C) Top panels: Co-IF used to detect SynT-I, SynT-II, and fetal endothelial cells using antibodies against MCT1, MCT4, and CD31, respectively. Bottom panels: Multiplex fluorescent ISH used to detect *Mct1* and *Mct4* mRNA. Arrowheads point to S-TGC. Arrows point to fetal nucleated red blood cell. Scale bar = 30 μm. (D) Diagram of the serotonin metabolic pathway. Serotonin (5-hydroxytryptamine, 5-HT) synthesis begins with hydroxylation of tryptophan, an essential amino acid, by tryptophan hydroxylase (TPH1 or TPH2), in a tetrahydrobiopterin (BH4)-dependent manner. This reaction is followed by a pyridoxal phosphate (PLP)-dependent decarboxylation by dopa decarboxylase (DDC, also called aromatic L-amino acid decarboxylase). 5-HT is cleared from cells after being converted to 5-HIAA by MAO-A and ALDH. Trp, tryptophan; 5-HTP, 5-hydroxytryptophan; DDC, dopa decarboxylase; PLP, pyridoxal phosphate; MAO-A, monoamine oxidase A; ALDH, aldehyde dehydrogenase; 5-HIAA, 5-hydroxyindoleacetic acid.

developmental ages, the placenta, which expresses both TPH1 and DDC in the syncytiotrophoblasts, produces 5-HT [5]. Most importantly, 5-HT produced by the placenta reaches the embryonic circulation and is transported to the forebrain prior to brain 5-HT axons reach this region [5]. Thus, the findings connect placenta function directly to influencing brain circuitry. Nonetheless, the specific cells responsible for 5-HT synthesis, degradation, and transport in the placenta are not known.

In the brain, in addition to TPH and DDC, several other molecules control the availability of 5-HT. Because 5-HT is hydrophilic in