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Developmental profile of neurogenesis in prenatal human hippocampus: An immunohistochemical study

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ABSTRACT

Hippocampus has attracted the attention of the neuroscientists for its involvement in a wide spectrum of higher-order brain functions and pathological conditions, especially its persistent neurogenesis in subgranular zone (SGZ). The development of hippocampus was intensively investigated on animals such as rodents. However, in prenatal human hippocampus, little information on the distribution of neural stem/progenitor cells, newly generated neurons and mature neurons is available and the timetable of a series of neurogenesis event is even more obscure. So in the present study, we aim at immunohistochemically providing more information on neurogenesis in prenatal human hippocampus from 9 weeks to 32 weeks of gestation. We found that the ki67-positive cells were always detected in hippocampus from 9 weeks to 32 weeks, with a peak at 9 weeks in cornu ammonis (CA) or 14 weeks in dentate gyrus (DG). At 9 weeks the nestin-expressing cells were distributed throughout the hippocampus, with concentrated immunoreactivity in intermediate zone (IZ), marginal zone (MZ), fimbria, and relatively sparse immunoreactivity in the ventricular zone (VZ) and hippocampal plate (HP). With development, the optical density (OD) and the number of nestin-positive cells decreased gradually. At 32 weeks, there were relatively more nestin-positive cells in DG than that in CA. About DCX-positive cells, they displayed a similar distribution as nestin-positive cells (immunoreactivity concentrated in IZ, MZ, fimbria and HP) and a dramatic decrease of OD or cell number density from 9 weeks on. NeuN-positive cells, with small nuclei, were firstly found in MZ and subplate of hippocampus at 9 weeks. After 14 weeks, many NeuN-positive cells extended from subplate into HP and the density of NeuN-positive cells peaked at 22 weeks. That the immunoreactivity for NeuN was the strongest and the nuclei were the biggest at 32 weeks suggests that the neurons reach maturity gradually. Therefore this study provides an important timetable of neurogenesis in prenatal human hippocampus for the clinicians in neuroscience or pediatrics.

Abbreviations: CA, cornu ammonis; DG, dentate gyrus; GL, granular cell layer; HP, hippocampal plate; IMZ, inner marginal zone; OD, optical density; IZ, intermediate zone; LV, lateral ventricle; ML, molecular layer; MZ, marginal zone; OMZ, outer marginal zone; PL, polymorphic layer; SLM, stratum lacunosum molecular; SO, stratum oriens; SP, stratum pyramidale; SR, stratum radiatum; VZ, ventricular zone.

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1. Introduction

The hippocampus is a representative of the archeocortex and plays an important role in learning and memory, emotion, and mood. Moreover, the subgranular zone (SGZ) in the dentate gyrus (DG) of the hippocampus is one of the important sites, where neurogenesis takes place throughout the lifespan of humans and other mammals (Raineteau et al., 2004). So the hippocampus has attracted the attention of many clinicians and neuroscientists for its involvement in a wide spectrum of pathological conditions, including epilepsy, intellectual disability, Alzheimer disease and others. Therefore, the development of hippocampus, especially its neurogenesis, is an important issue to be clarified for neuroscientists.

It is generally acknowledged that neurogenesis mainly includes coordination of neural stem/progenitor cells proliferation, neural lineage differentiation, migration and development of neuronal features. In studies, some particular antigen markers have been used for about two decades to identify differential kinds of cells, related to neurogenesis (Lyck et al., 2008; von Bohlen Und Halbach, 2007, 2011). Neural stem/progenitor cells can express several particular proteins as antigen markers: nestin, GFAP, Sox2, Musashi-1, Pax6 and so on (von Bohlen Und Halbach, 2007, 2011). Thereinto, nestin is a member of the intermediate filament protein family and this marker is widely used to indicate neural stem/progenitor cells in study (Lillien, 1998; Mignone et al., 2004). The neural stem cells generate a large number of daughter cells by means of proliferation and differentiation. The expression of the endogenous proteins Ki67 and proliferating cell nuclear antigen (PCNA) are strictly associated with cell proliferation (von Bohlen Und Halbach, 2007, 2011; Scholzen and Gerdes, 2000; Eisch and Mandyam, 2007). Ki-67 is thought to represent a more reliable marker for identifying cells that re-enter the cell cycle than PCNA (Kee et al., 2002). Identification of immature neurons can be done with DCX, Tbr2, NeuroD, PSA-NCAM, Tuj-1, etc. (von Bohlen Und Halbach, 2007, 2011). DCX is a brain-specific microtubule-associated protein and expressed by migrating, differentiating neuroblasts (Bai et al., 2003). There are also several other markers, including NeuN (Neuronal Nuclei), NSE and calbindin-D, that are expressed at a mature stage of neurons (Christie and Cameron, 2006). NeuN is a protein "marker" detected exclusively in post-mitotic neurons (Mullen et al., 1992). Immunohistochemically, NeuN is detected only in mature neurons and is absent from neural progenitor, glia, oligodendrocytes and astrocytes (Sarnat et al., 1998; Korzhevskii et al., 2006).

Therefore, the temporal and spatial patterns of neurogenesis in developing brain have been exclusively studied in rodents, mainly in rats and mice (Danglot et al., 2006; Khalaf-Nazzal and Francis, 2013). In mice, the CR cells in marginal zone (MZ) are among the first neurons to be generated at E10 in cerebral cortex, including hippocampus (del Río et al., 1995). The pyramidal neurons of cornu ammonis (CA1) are born at E10 and peak at E15 (Angevine Jr., 1965). Although CA3 neurogenesis starts 1 day later compared to CA1, it peaks 1 day earlier, at E14 (Stanfield and Cowan, 1979; Bayer, 1980a). However, neurogenesis in the DG extends over a longer period of time. Starting as a proliferation of ventricular zone (VZ) neuroepithelial cells early at E10 (Deguchi et al., 2011), it peaks at E16, and continues to reach the second peak until the first postnatal week (Bayer, 1980a,b). Few DG cells are produced around E10 (Deguchi et al., 2011). Early studies using radioactive 3H-thymidine labeling showed that many granule cells were born later at E16, and an accumulation of cells became visible in the DG region at E18 (Angevine, 1965).

In term of the development of prenatal human hippocampus, its morphogenesis has also been well explained in the literature by diagrams showing progressive infolding of the fetal DG, CA, subiculum, and parahippocampal gyrus around the progressively smaller hippocampal sulcus (Baker et al., 1992; Sasaki et al., 1993). These diagrammatic representations are based on the histologic study reported by Humphrey (Humphrey, 1967). Then later studies with magnetic resonance imaging resembled the results in histology (Bronen and Cheung, 1991; Kier et al., 1997).

The cytoarchitecture, myeloarchitecture, neuronal morphology and neuronal cytoskeletonin of human fetal hippocampus were studied in literatures (Arnold and Trojanowski, 1996a,b). The development of the cells expressing nitric oxide or S100ß in the prenatal human hippocampal formation was revealed with immunohistochemical method in further studies (Yan and Ribak, 1997; Chan et al., 2003). However, in prenatal human hippocampus, not much information on the distribution of neural stem/progenitor cells, newly generated neurons and mature neurons is available and the timetable of a series of neurogenesis event is even more obscure. So in the present study, we aim at immunohistochemically providing more information on neurogenesis in prenatal human hippocampus from 9 weeks to 32 weeks of gestation with some above mentioned, acknowledged antigen markers.

2. Materials and methods

2.1. Human fetus brain samples

A total of 45 deceased fetuses were obtained from spontaneous, elective or medical termination of pregnancy. The whole protocols were approved by the Ethics Committee of Medical College of Xi'an Jiaotong University (No. 2012-225) and followed the guidelines of the Declaration of Helsinki. The specimen collection was conducted in accordance with the guidelines of National Institutes of Health. Informed consent was obtained from the families concerned before specimen collection. More information of the cases was summarized in Table 1. Gestational age was estimated from the last menstrual period, as well as foot length and crown-rump length. There was no clinical history of family linked neurologic or psychiatric disorders in any of these cases. The reasons for the stillbirth were not ascertained by post-mortem examination. The interval between death and tissue fixation was usually ≤12 h. It should be noted that many of the discarded fetuses had a longer

Table 1
Summary of cases.

Case	Age	Gender	Classification
1	9	Not applicable	Elective
2	9	Not applicable	Spontaneous
3	9	Not applicable	Spontaneous
4	10	Not applicable	Elective
5	10	Not applicable	Spontaneous
6	10	Not applicable	Spontaneous
7	11	Not applicable	Elective
8	11	Not applicable	Spontaneous
9	11	Not applicable	Spontaneous
10	14	Male	Elective
11	14	Male	Spontaneous
12	14	Male	Medical
13	14	Female	Spontaneous
14	15	Male	Medical
15	15	Female	Elective
16	15	Female	Spontaneous
17	15	Male	Spontaneous
18	16	Female	Spontaneous
19	16	Female	Spontaneous
20	22	Female	Elective
21	22	Female	Spontaneous
22	23	Male	Medical
23	23	Female	Elective
24	23	Female	Medical
25	24	Male	Medical
26	24	Female	Medical
27	24	Male	Medical
28	24	Female	Elective
29	32	Male	Stillborn
30	32	Female	Stillborn
31	33	Male	Stillborn
32	34	Female	Stillborn
33	34	Male	Stillborn
34	36	Female	Stillborn

Age refers to gestational age in weeks.