Migration and accumulation of primordial germ cells of early embryo in quail

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Abstract

In this research, we have identified primordial germ cells (PGCs) in quail embryo using Quail Hemangioblastic Lineage (QH1) monoclonal antibody analysis. Quail PGCs originated from the opaca of unincubated blastodisc, and then transferred to the pellucida and the germinal crescent. At 27 hours post-incubation, a few PGCs first appeared in blood vessels of the pellucida, while many PGCs accumulated at 36 hours post-incubation. The PGCs scattered or clustered from head to omphalo mesenteric area, then transferred to the pellucida and the germinal crescent via the lower layer at stage 4 (18 hours post-incubation), and then differentiated into gametes (Fujimoto et al., 1976). The origin, migration and location of the PGCs were well reported in chicken, while few in quail. Distribution of quail PGCs before migration to the germinal crescent raised much dispute. Dubois and Cuminge (1979) predicted that most PGCs were distributed in the embryonic tail area during unincubated stage, while Fargeix (1989) pointed out that PGCs tended to migrate to the head area, including the opaca, but surpassing the germinal crescent. Another valuable issue is the non-symmetrical distribution of PGCs in the avian reproduction region. In birds, the left sexual gland holds more PGCs than the right, which was first observed by Firket (1914), then certified in various species (Didier and Fargeix, 1976). However, its mechanism and distribution difference between various species are so far unknown. In addition, there are many recent reports on transgenic chicken production by transfection of PGCs (Didier and Fargeix, 1976). The short developmental period of quail embryo is advantageous to operate comeptently. The present study aims to understand the origin, migration and location of the PGCs of early embryo in quail, intending to clarify the puzzles and germ cell determination and differentiation. Furthermore, as an important animal model, a full understanding of the developmental mechanism in quail would be helpful to the improvement of avian transgenic technology and the avian production.

Materials and methods

Fertilized eggs and animal care

Japanese Quail (Coturnix japonica) belongs to Aves, Califormias, Phasianidae, Coturnix, from which 39 fertilized eggs were obtained by natural mating. The quail population was maintained with standard management program at Jiangyan quail breeder farm, Jiangsu province, China.

QH1 antibody preparation

The monoclonal antibody QH1 was bought from Developmental Studies Hybridoma Bank developed under the auspices of the NICHD and maintained by the Iowa University, Dep. of Biological Sciences Iowa City, IA, USA. The fluorescence marked Goat Anti-mouse IgG was bought from Rockland (Dakewe Biotech CO., LTD., Beijing, China).

Preparation of embryo samples

The hatched quail eggs were incubated at 38°C and a relative humidity of 60% (Incubator 4820, Wuxi Mingfeng Incubation Technology, China). The germinal disks at 0 to 45 h were randomly obtained using 6 samples for each group. The blastodiscs were cut out with the area opaca and carefully cleaned of vitellus, paying deep attention to place them perfectly flat, avoiding folds. Stage was classified according to Eyal-Giladi and Kochav (1976) system (<12 hours) and Hamburger and Hamilton (1951) system (>12 hours), while later stages were defined from the number of somites.

Immunochemical staining

Quail embryos were fixed for 3 hours in 4%
paraformaldehyde. Embryos were washed twice with phosphate-buffered saline (PBS) at room temperature, and then dehydrate the embryos in 100% methanol for 1 h at -20°C. Specimens were dehydrated through a graded ethanol series. Thereafter, rehydration in 100%, 95%, 75% ethanol in 4°C, 15 to 20 min each times, and 50% ethanol at room temperature. The embryos were stained using Goat Anti-mouse fluorescence IgG, then washed twice with PBS, and blocked at 4°C overnight with blocking solution, additionally followed by incubation with 1:100 QH1 at 4°C overnight, washed three times with PBS at 4°C, and incubated with 1:200 Goat Anti-mouse IgG at 4°C overnight away from light. This antibody was withdrawn and washed three times with PBS at 4°C for 5 min each.

Then the preparations were dehydrated through graded ethanol solutions, in 50%,

Figure 1. Immunohistochemical staining results of early embryonic quail. (A) Whole embryo at 12 h post-incubation (×40), scale bar: 200 μm. (B) Local enlargement observation of the embryo at 12 h post-incubation (×400), scale bar: 20 μm. (A, B) XII-XIII EG K (stages were classified according to Eyal-Giladi and Kochav, 1976): stage blastodisc reacted with QH1. PGCs scattered throughout the blastodisc, where mostly distributed in the boundary between area opaca and area pellucida. Single cell is large and round. (C) Whole embryo at 19 h post-incubation (×40), scale bar: 200 μm. (D) Local enlargement observation of the embryo at 19 h post-incubation (×400), scale bar: 100 μm. (C, D) Head process stage blastodisc reacted with QH1. Germinal crescent near a visible fibrous band (arrows). (E) Whole embryo at 23 h post-incubation (×40), scale bar: 200 μm. (F) Local enlargement observation of the embryo at 23 h post-incubation (×400), scale bar: 100 μm. (E, F) Third somite stage blastodisc reacted with QH11. Anterolateral region of the area opaca. (G) Whole embryo at 27 h post-incubation (×40), scale bar: 200 μm, (H) Local enlargement observation of the embryo at 27 hours post-incubation (×400), scale bar: 100 μm. (G, H) Seventh somite stage blastodisc reacted with QH1. A few PGCs distributed in the area of opaca vascular network. (I) Whole embryo at 36 h post-incubation (×40), scale bar: 200 μm. Tenth somite stage blastodisc reacted with QH1. At this stage, most PGCs distributed in the area of both sides of the opaca vascular network. (J) Whole embryo at 45 hours post-incubation (×40), scale bar: 200 μm. Eighteenth somite stage blastodisc reacted with QH1. PGCs surrounded in the heart, big blood vessels and head of quail embryo, mostly in the blood vessels of the head mesenchymal.
70%, 95% ethanol 20 min each time, and in 100% ethanol 20 min twice. QH1 antibody was used to identify the PGCs. The preparations were treated with toluene and then observed under fluorescent microscope (BX51T-32F01-FLB3, Olympus, Japan), and the labelled cells were counted under a microscope.

**Results**

**Migration, accumulation and distribution of PGCs at different stages**

Cells with green fluorescence were observed in pellucida area at different stages, which resulted from QH1 marker. These cells were PGCs, with certain characteristics: big size (12-25 μm), round or egg-shaped with pseudopodium. After 45 h of incubation, the interference of endothelial system would make difficult the observation of PGCs, so we observed germinal disks 0 to 45 h post-incubation. The results were as follows. Figure 1 and 2 showed the immunohistochemical staining results of early embryonic quail and blastodisc reacted with PBS (a control group). The results are described as followed.

**Unincubated embryonic disk**

The PGCs were distributed in few specimens, which almost distributed in the area opaca, adjoining to the area pellucida (stages X-XI, EG and K classified according to Eyal-Giladi and Kochav, 1976). They scattered or clustered by two and three.

**Embryonic disk at 12 h post-incubation**

The PGCs were distributed in the area pellucida by twos and threes at 12 h post-incubation shown in Figure 1A, B (stages XII-XIII, EG and K). The PGCs scattered the whole embryonic disk, but focused on the adjoining area of pellucida and opaca, with big and round cells, as indicated in Figure 1A, B with arrows.

**Embryonic disk at 18 h post-incubation**

The adjoining area of pellucida and opaca were getting thicker, and a column-shaped primitive streak about the whole length of pellucida formed in the center of the area pellucida; and a great number of PGCs were observed in the pellucida at the fore end of the primitive streak (primitive streak stage).

**Embryonic disk at 19 h post-incubation**

The PGCs scattered or clustered with 9 cells gathered at most. They were distributed mainly in the germinal crescent of pellucida and only a few in the opaca, as shown in Figure 1C, D (head process stage).

**Embryonic disk at 23 h post-incubation**

QH1 positive cells increased as expected; most of the PGCs were located in the front half of germinal disks, only a few separate cell populations in the tail area. The cells in the front still focused on the germinal crescent, as shown in Figure 1E, F (third somite).
Embryonic disk at 27 h post-incubation

PGC populations were distributed sparsely, and few cells were observed in blood vessel in pellucida area, as shown in Figure 1G, H (seventh somite).

Embryonic disk at 36 h post-incubation

Left and right omphalomesenteric were formed on the side of embryo heart, and capillary network also formed in the pellucida area. Blood islands have spread in all the opaca area, and linked with capillary network in the pellucida area. Many PGCs clustered in the blood vessel in the pellucida area, as shown in Figure 1I (tenth somite).

Embryonic disk at 45 h post-incubation

The heart region was differentiated into atria and ventricle at 45 h post-incubation (eighteenth somite). PGCs interspersed among the pellucida area in front of the embryo head and near the middle and tail area of embryo. Many PGCs clustered in mesenchymal blood vessel in the embryo head (Figure 1J). PGCs scattered from head to omphalomesenteric, mainly in the mesenchymal area in the head (Figure 1J).

The PGCs amounts in different stages of quail embryo

The size of PGCs population increased significantly (P<0.05) from stage XII (12.8±4.82 μm) to primitive streak stage (106.7±8.74 μm) and from head process stage (95.8±19.74 μm) to tenth somite stage (199.4±19.97 μm). The amount of PGCs varied at different stages with two peaks, primitive streak stage (18 h post-incubation) and tenth somite (36 h post-incubation) (Table 1).

Table 1. The PGCs amounts in different stages of quail embryo.

| Stages Incubation time | Number | PGCs numbers | Mean±SD |
|------------------------|--------|--------------|---------|
| X-XI EG K’ Laying      | 6      | nd           |         |
| XII-XIII EG K 12 hours | 5      | 11, 9, 21, 13, 10 | 12.8±4.82|
| Primitive streak HH** 18 hours | 3      | 114, 109, 97 | 106.7±8.74|
| Head process stage 19 hours | 6      | 101, 112, 73, 89, 123, 77 | 95.8±19.74|
| Third somite 23 hours 5 | 5      | 183, 154, 121, 179, 104 | 148.2±34.95c|
| Seventh somite 27 hours | 3      | 172, 165, 184 | 173.7±38.61c|
| Tenth somite 36 hours 7 | 7      | 205, 191, 168, 231, 206, 187, 211 | 199.4±19.97c|
| Eighteen somite 45 hours 4 | 4      | 131, 136, 129, 101 | 124.2±15.78bc|

*abc Means within a column lacking a common superscript differ (P<0.05). *Stages classified according to Eyal-Giladi and Kochav (1976) (EG K). **Stages classified according to Hamburger and Hamilton (1951) (HH). nd: not determined.

2) Do the PGCs have non-symmetrical distribution in the reproduction region?

Our study supported other authors’ findings (Fargeix, 1969; Ginsburg, 1997), indicating that before migration to the germinal crescent (12 h post-incubation), PGCs translocated the hypoblast and were carried into the germinal crescent mainly by the hypoblast. As for the problem of the non-symmetrical distribution of PGCs in the reproduction region, different avian species demonstrated different modes. Fargeix (1969) found the non-symmetry in duck and Clawson and Domm (1969) also found the same in chicken. However, Pardanaud et al. (1987a) found that the distribution of PGCs was highly erratic and varied randomly between the blastodisc. In this paper, the PGCs were randomly distributed in the germinal disk.

The numbers of PGCs are generally varied at different stages. Many reports focused on it, but whether the numbers of PGCs increase or not from primitive streak stage to seventh somite stage is still unclear. Clawson and Domm (1969) found there was no obvious increase of PGCs in chick from primitive streak stage to fourth somites period, but obvious increase at the stage of seventh somite. David (1975) found obvious increase of PGCs from primitive streak stage to seventh somites period, in chick and quail. Pardanaud et al. (1987a) found the average number of quail PGCs did not increase significantly by time passing between the early primitive streak stage and the seventh somite stage. Li et al. (2000) found the peak of PGCs clustering at the stage 5, 11, 13 and 17 (22, 44, 52 and 62 h post-incubation). Li et al. (2003) found that fluorescent marked PGCs moved to embryonic germinal base 48 h post-incubation. Ye et al. (2002) proposed that in duck the peak of PGCs occurred at stage 16 (80-89 h post-incubation). In this study, we found obvious increase of PGCs in quail from unincubation to primitive streak stage and from primitive streak stage to tenth somite stage, and therefore we consider that from the primitive streak stage and the seventh somite stage the numbers of PGCs are also increasing.

Conclusions

Quail PGCs originated from the opaca of unincubated blastodisc, and then transferred to the pellucida and the germinal crescent. The PGCs scattered or clustered from head to omphalo mesenteric and mainly settled down in the mesenchymal blood vessels of head at 45 h post-incubation. It is concluded that the PGCs...
scattered in the head area before migration to the germinal crescent and distributed randomly and interspersedly in the both gland. The number of PGCs varied at different stages with two peaks, primitive streak stage (18 h post-incubation) and tenth somite (36 h post-incubation).

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