Scanning Electron Microscopic Study on Human Primordial Germ Cells during the Migration Period

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Summary. The surface topography of human primordial germ cells (PGCs) during the migration period was observed by scanning electron microscopy, paying special attention to the association of PGCs with somatic cells. PGCs in the stationary phase of their migration were spherical in shape and measured 12 μm in diameter. The cell surface was generally smooth with some microvilli. On the other hand, PGCs in the moving phase revealed an indefinite shape and often possessed pseudopodia. Their surface was somewhat undulating and microvilli were rarely found. PGCs were often seen to make contact with neighboring mesenchymal cells during their migratory course, especially at the root of the dorsal mesentery or other areas near the gonads. Two types of contact were observed: direct adhesion of a mesenchymal cell body to a PGC, and indirect adhesion by cytoplasmic protrusions from a mesenchymal cell to a PGC. In the latter case, three types of protrusions, i.e. lobopodia, filopodia, and lamellipodia, were observed. These contacts between PGCs and somatic cells may be related to the mechanisms of migration of PGCs.

Primordial germ cells (PGCs) in human embryos originate in and separate from the endoderm at the posterior region of the yolk sac, and finally migrate to the gonadal anlage through the dorsal mesentery by amoeboid movement (Witschi, 1948; Fujimoto et al., 1977). Studies on the migration and morphology of PGCs in developing human embryos have advanced at the light microscopic (LM) level (McKay et al., 1953; Pinkerton et al., 1961; Falin, 1969; Fuyuta et al., 1974). Recently, transmission electron microscopy (TEM) was used to reveal the ultrastructure of PGCs from their time of appearance in the endoderm to their entry into the gonadal anlage in humans (Fukuda, 1976; Fujimoto et al., 1977; Miyayama et al., 1977). Based on LM and TEM studies, human PGCs are morphologically identifiable by their extremely large cell body, vesicular nucleus, and condensed nucleolus, and by the large amount of glycogen and lipid. Moreover, it has been shown that PGCs in the migratory course contacted neighboring mesenchymal cells, and
that intercellular junctions, such as desmosomes, were often present in this contact area (Fujimoto et al., 1977; Miyayama et al., 1977).

It is important for analyzing the mechanisms of migration of PGCs to know the surface topography of these cells and the three dimensional relationships between PGCs and the surrounding mesenchymal cells during the course of migration. For this purpose, PGCs migrating to the gonads in human embryos were observed by scanning electron microscopy (SEM).

Materials and Methods

The materials used for this investigation were early human embryos obtained by legal abortion, which were estimated to be at the 5th to the 6th week of development. The embryos were fixed with 3% glutaraldehyde (0.05 M cacodylate buffer, pH 7.4) for 3 hrs to overnight at 4°C. Subsequently, they were sectioned transversely into several thin pieces with a razor blade at the level of the gonads. The sectioned specimens were postfixed with 1% osmic acid (0.05 M cacodylate buffer, pH 7.4) for 1 hr at 4°C, washed in the same buffer, dehydrated in a graded series of ethanol and finally dried by the critical point method using liquid CO₂. The dried specimens were mounted sectioned-surface up on aluminum stubs with silver paint. They were sputter-coated with Au-Pd and observed under a JSM-50A scanning electron microscope operated at 15 kV.

After examination by SEM, specimens which contained PGCs in their sectioned-surfaces were taken from the stubs, infiltrated with propylene oxide and embedded in epoxy resin. Each specimen was cut to 1 μm in thickness in parallel with the sectioned-surface and stained with toluidine blue. The identity of the cells observed by SEM was then confirmed by LM.

Results

As shown in Figure 1, PGCs are first recognized in the endoderm at the posterior region of the yolk sac at the 4th week of development. They then separate from the endoderm and migrate to the gonadal anlage by active movement through the dorsal mesentery during the 5th week.

In cross sections of plastic-embedded embryos at the 5th week, many PGCs were found in the mesenchyme in the vicinity of the gonadal anlage (Fig. 2). These PGCs were mostly round in shape but a few were deformed. This seems to indicate that PGCs do not always move, but often pause in their migratory route, taking on a round shape.

By SEM, some PGCs showed a spherical profile, suggesting the stationary phase, and measured about 12 μm in diameter (Fig. 3). The surface of the PGCs was generally smooth, but possessed several microvilli. These measured 120 nm in diameter and their length was about 2.0-2.5 μm, although shorter ones were also found.

On the other hand, some PGCs were found to be indefinite in shape, and larger than the neighboring mesenchymal cells. They were sometimes elongated or contained pseudopodia, suggesting the moving phase. In the latter case, the pseudopodia may indicate the direction of cell movement (Fig. 4). The surface of the migrating PGCs displayed undulations or folds. These structures could arise from the conspicuous alteration of the cell surface in locomotion. The mesenchymal cells were small and also showed an irregular shape with several cytoplasmic processes. Their surface was, however, smooth with few microvilli.

PGCs in the course of migration to the
gonads were frequently observed to be in close contact with surrounding mesenchymal cells. Such PGCs had spherical profiles, and appeared to pause temporarily. In almost all cases, the nature of the contact between the two kinds of cells was such that a mesenchymal cell body or part of it adhered to a PGC. The contacts could be subdivided into two types; direct adhesion of the mesenchymal cell body to the PGC, and indirect adhesion by cytoplasm elongated from the mesenchymal cell. We designate these two types as direct and indirect adhesion, respectively.

In the "direct adhesion type", the PGC appeared to be wearing a mesenchymal cap, because the mesenchymal cell was considerably smaller than the PGC (Figs. 5 and 6). In this case, the mesenchymal cell exhibited a plain profile, possessing no microvilli and pseudopodia. The thin peripheral cytoplasm of this cell was adherent to the PGC.

On the other hand, the "indirect adhesion type" was composed of adhesions by different cytoplasmic processes from mesenchymal cells, consisting of lobopodial, filopodial and lamellipodial protrusions. Figures 7 and 8 show the adhesion by several kinds of cytoplasmic processes from surrounding mesenchymal cells to the PGC. Such cytoplasmic protrusions were composed of large lobopodial protrusions and fine filopodial protrusions. In addition, intermediate-sized processes were also found. The large protrusions measured 1 μm in diameter and the distal end was somewhat wide; the fine ones were under 100 nm in diameter.

The lamellipodial protrusion revealed the more striking features (Figs. 9 and 10). This structure stretching from the mesenchymal cell was seen to creep on the surface of PGC. The distal end of the lamellipodium was ramified. In this type, the cytoplasm of the mesenchymal cell was often stretched thinly, so that the site of the nucleus was recognized as a swelling.

**Discussion**

Investigations by LM and TEM have demonstrated the fundamental characteristics of human PGCs. Their cell body is large, their nucleus is also large and round, their nucleolus is strongly condensed, and their cytoplasm contains large amounts of glycogen and lipid. These morphological characteristics of human PGCs basically resemble those in the PGCs of other vertebrates. It is easy therefore to identify human PGCs by LM. In fact, PGCs after observation by SEM were reexamined and identified by LM in the present study.

Only a few SEM studies on PGCs have been described. Ukeshima and Fujimoto (1978) observed chick PGCs by SEM and reported the surface morphology of those from the original site in the yolk sac endoderm to the gonadal site after migration. Lee et al. (1978) also observed chick PGCs just after their separation from the endoderm. In amphibians, Heasman and Wylie (1978) reported SEM figures for Xenopus PGCs in relation to their locomotion in vitro. However, PGCs in humans have not previously been observed by SEM. This is due to the fact that human embryos are relatively difficult to obtain and only PGCs in a sectioned-surface of the embryo can be observed. In the present study, the surface morphology of human PGCs was investigated for the first time.

The size of PGCs observed by SEM was somewhat smaller than that by TEM (Fujimoto et al., 1977). This was probably due to shrinkage during critical point drying.

The surface topography of human PGCs resembles to that of chick PGCs in
presenting a smooth surface with some microvilli (Ukeshima and Fujimoto, 1978). However, differences are seen between chick and human PGCs. In chick PGCs, after separation from the endoderm, blebs are observed on their surface, which seem to reflect the sites of yolk granules. Such blebs are also found in amphibian PGCs (Heasman and Wylie, 1978). However, no blebs were observed in human PGCs in the present study.

Close association of human PGCs with neighboring somatic cells during the migration period was emphasized by Fujimoto et al. (1977) based on TEM studies, and even tightly connected structures such as desmosomes or intermediate junctions, were found between these two types of cells. They considered that the contact observed between the PGCs and somatic cells was related to the migration mechanism of PGCs. In this study, PGCs present in the mesenchyme near the gonads were observed to be in contact with neighboring somatic cells, and such PGCs did not show amoeboid features in most cases. This suggests that PGCs may stop migrating for contact with mesenchymal cells, and such PGCs did not show amoeboid features in most cases. This suggests that PGCs may stop migrating for contact with mesenchymal cells. In the present study, SEM revealed that two types of contact existed between PGCs and somatic cells. In the case of the "direct adhesion type", the mesenchymal cells had their cell body in direct contact with PGCs. By TEM, Miyayama et al. (1977) reported the case of a PGC being attached to a semilunar mesenchymal cell. This appears to correspond to the direct adhesion type in our present study. In the case of the "indirect adhesion type", three forms of cytoplasmic protrusions for adhesion, i.e. lobopodia, filopodia, and lamellipodia, were observed. However, these structures could not be distinguished by TEM (Fujimoto et al., 1977; Miyayama et al., 1977).

It is unknown whether the above adhesion patterns have different functions or represent steps in the adhesion process to a PGC. However, it is considered that the structures for adhesion between PGCs and mesenchymal cells, especially the lamellipodial adhesions, are intimately involved in the migration mechanisms of PGCs.

References


Explanation of Figures

Plate I

Fig. 1. Schematic drawing showing the migration course of PGCs from the hindgut endoderm to gonads at each period of development. 4W: separation period. 5W: migration period. 6W: settlement period. Mt: mesentery. GR: germinal ridge.

Fig. 2. Cross section through the developing gonadal area of a 5th-week embryo. PGCs are readily identifiable by their morphological characteristics. One PGC (large arrow) shows amoeboid features and others are round in shape (small arrows). 1 μm plastic section stained with toluidine blue. Ep: epithelium of germinal ridge. Ao: dorsal aorta. ×600
Plate II

Fig. 3. A PGC rounding in the mesenchyme at the root of the mesentery in a 5th-week embryo. This PGC appears oval in shape and the surface is smooth with a few microvilli. Inset: light micrograph of the same PGC. Toluidine blue staining. ×5,000

Fig. 4. A PGC found in the mesentery of an early 5th-week embryo. The cell is extending its cell body for migration. Many swellings and some undulations of the cell surface are seen in this PGC. ×4,000
Plate III

Fig. 5. A PGC found in the root of the mesentery of a 5th-week embryo. A mesenchymal cell (Ms) is in direct contact with the PGC (arrow). The surface of these two types of cells is smooth. $\times 3,000$

Fig. 6. Enlargement of part of Figure 5. The edge (arrows) of the mesenchymal cell (Ms) adheres to the PGC (GC). $\times 6,000$
Plate IV

Fig. 7. A PGC (GC) in contact with surrounding mesenchymal cells by various cytoplasmic processes (arrows). The intercellular space around the PGC is relatively wide. 5th-week embryo. Inset: light micrograph of the same PGC from a 1 μm section stained with toluidine blue. ×4,000

Fig. 8. Enlargement of part of the cell in Figure 7. The distal end of a large lobopodial protrusion (large arrow) is seen to adhere to the PGC (GC). Fine protrusions also extend from the mesenchymal cell (small arrows). The PGC has several microvilli. ×9,000
Plate V

Fig. 9. Lamellipodial adhesion in a 5th-week embryo. A large lamellipodium (L) from an upper mesenchymal cell (Ms) adheres to a PGC. Part of it (arrow) extends to a lower mesenchymal cell (Ms). ×3,000

Fig. 10. Enlargement of part of Figure 9. As the lamellipodium (L) becomes thin, folds of the surface of the PGC are visible through it. Arrows: margin of the lamellipodium. ×8,000