The Origin of Endoderm: Transmission Electron Microscopic Point of View

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ABSTRACT

Objective: To show the transmission electron microscopic (TEM) evidence to confirm that the endoderm originates from the epiblast of the primitive streak or from other sources.

Methods: 60 fertilized Leghorn hen's eggs were used in this study by incubating the eggs for about 18-27 hours at 38°C, then the chick embryos of the primitive streak stage to 7-somite stage were further processed for routine TEM study at the region of the primitive streak.

Results: The epiblast proliferates and accumulates to form the primitive streak at the midcaudal of the embryonic disc from 18-27 hours incubation which corresponds with the early third week of the human embryo. TEM evidence shows that the epiblast at the primitive streak is the stratified columnar type of epithelium while the hypoblast is the simple squamous and the mesoderm cells are irregular in shape. The process of gastrulation begins with the formation of the filopodia of the epiblast by numerous protrusions of the plasma membrane from lateral side of the cell. These structures initiate the separation of the contacted cells. The deepest epiblast cells separate first while the superficial epiblast cells exhibit the desmosome between the adjacent cells. The separated epiblast cells are bottle-shaped with numerous filopodia and gradually change the shape into round or oval cells which migrate in the space between the epiblast and hypoblast. Some of these migrate to the hypoblast and contact with the hypoblast, the mesoblasts lose the filopodia and gain more close contact to the hypoblasts which become a very thin sheet of cells. The facing cell membrane later gradually disappears and the mesoblast then occupies the region of pre-existing hypoblast. There is no evidence that the mesoblast displaces the pre-existing hypoblast laterally to form the extraembryonic endoderm.

Conclusion: These are TEM evidences that the epiblast of the primitive streak separates and migrates to form the mesoblast and some contact with the hypoblast. The later process appeared to reveal that the mesoblast compresses the hypoblast until the facing plasma membrane disappears and occupies the region of the pre-existing hypoblast.

Keywords: Ectoderm, mesoderm, endoderm transmission electron microscopy

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Gastrula is the human embryo of the fifteenth day after conception. It comprises of 3 germ layers, the ectoderm, the mesoderm and the endoderm. In day 15, the embryo exhibits an important structure, the primitive streak. This structure originates from the proliferation of the epiblast cells at the midline of the caudal part of the embryonic disc and appears as a drum stick with a pedal and a round oval end, the primitive knot. Later in the sixteenth day, there is a groove appears at the midsagittal plane of the primitive streak. This is because the epiblast cells of the primitive streak invaginate and migrate inside to form the mesoderm, the middle layer of the trilaminar embryonic disc. At the same time, the epiblast cells at the primitive knot also proliferate and migrate inside to form a rod-shaped structure, the notochord. The epiblast in this way gives rise to the mesoderm and the notochord, while the mesoderm migrates everywhere in the middle layer except the oral and cloacal membranes. The notochord is the primitive axis and strengthens the embryo and will eventually degenerate except for a small part which gives rise to the nucleus pulposus of the intervertebral disc.[1-3] Recently, it is believed that the

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epiblast of the primitive streak also gives rise to the endoderm. This is explained by light microscopy and immunocytochemistry that epiblast cells migrate to form the middle layer, while some of the cells also migrate deep down and press beside the pre-existing hypoblast cells and push them laterally.[4-7] Finally, the hypoblast is pushed away from the intraembryonic area and replaced by the epiblast of the primitive streak.[8-11]

Nowadays there is still no data about the transmission electron microscopic (TEM) study of the epiblast, the mesoblast and the hypoblast, although these were demonstrated by using scanning electron microscopy (SEM) as shown in Brown and Sander (1991) and also there is no ultrastructural proof about how the epiblast cells give rise to the endoderm. It is therefore interesting to study about the transmission electron microscopic structures of the epiblast, the mesoblast and the hypoblast and to see is there really the replacement and displacement of the hypoblast by the epiblast of the primitive streak.

MATERIALS AND METHODS

60 fertilized Leghorn hen's eggs which were purchased from the Faculty of Agriculture, Kasetsart University were used in this study by incubating the eggs for about 18-27 hours at 38°C to get the embryos of the primitive streak stage to 7-somite stage. The opening procedures started with cracking on the edge and cutting the shell around the equatorial line. The embryo usually lies on the top of the yolk which was cut around the area opaca with the fine pointed scissors. The embryo was removed with a flat spoon and agitated in a Petri dish with warm normal saline solution to remove adherent yolk. The embryos were fixed in 2.5% glutaraldehyde in 0.075 M cacodylate buffer, pH 7.3 for 5 minutes. After being washed in the same buffer solution, the specimens were postfixed in 1% osmium tetroxide for 1 hour at 4°C. Following dehydration with ethanol, they were embedded in a mixture of Epon-Araldite. Sections of 1 µm were stained with toluidine blue and observed by light microscope to confirm the area of satisfaction, which is the primitive streak, which usually lied at the caudal end of the embryonic disc. Ultrathin sections were serially cut, collected by copper grids and stained with uranyl acetate and lead citrate for 40 minute in each dye. The sections were examined with a transmission electron microscope.

RESULTS

At the primitive streak region, the embryonic disc comprises 3 germ layers, the epiblast, the mesoblast and the hypoblast. The epiblast is the stratified columnar epithelium (Fig 1). Much of the cell is the columnar-shaped cell and there are about 2 to 4 layers. The surface layer is mostly columnar while some cells are peg-shaped (Fig 1) which are characterized by broad apex and slender deep part. The deeper layer remains columnar with the upper part of the cells becoming narrower and are known as bottle-shaped cell. The deepest layer begins to change its cell shape to be round or oval. These cells start to migrate to form the mesoblast cells. All epiblast cells are generally surrounded by plasma membrane within with the cytoplasm and prominent euchromatic nuclei. Each nucleus is surrounded by a double membrane, and usually locates at the base of the cell (Fig 2). The plasma membrane of the epiblast can be divided into three surfaces, the upper, lower and the lateral surfaces. The upper surface of the cell faces with the vitelline membrane and the egg white. This surface has numerous pseudopodia projecting upward for a phagocytotic purpose. The lateral surface connects the neighbouring cells. In the bottle-shaped and peg cells there are numerous, long slender plasma membranes and cytoplasm extends from the lateral surfaces known as filopodia, the threadlike projections which do not serve any phagocytotic purpose, but for are used for initiation of cell separation. The epiblast cells at the deeper layer also possess filopodia which extend from all surfaces, but become shorter. The nuclei of the epiblast cells are euchromatic while other organelles are of embryonic type such as small round mitochondria, and a short cistern of rough endoplasmic reticulum. The most abundant organelles in the cytoplasm are several sizes of vacuoles, some are clear, but some are not, and these are the vacuoles of the nutritive materials which cells phagocytosed from outside. The deepest cells of the epiblast, after acquiring several filopodia, become separate from adjacent cells and change their shape to round or oval then migrate away to form the mesoderm. The mesoblast cell still acquires filopodia, migrates laterally and ventrally associates with the hypoblast. The nucleus of the mesoblast is large and euchromatic with other organelles such as mitochondria, rough endoplasmic reticulum and several vacuoles. The hypoblast cells arrange themselves as the simple squamous epithelium with flat and small nucleus, with abundant vacuoles, while the other organelles are difficult to identify (Fig 5). The mesoblast cells which migrae from the epiblast of the primitive streak acquire the amoeboid movement. Some cells reach the hypoblast and contact, and at the same time some hypoblast cells separate and the mesoblast cell replaces them. At the area of some contacts, the hypoblast cells have not already separated, so the mesoblast cells compresses the hypoblast until the cytoplasm of the hypoblast at this area become very thin and the mesoblasts will eventually replace the pre-existing hypoblast.

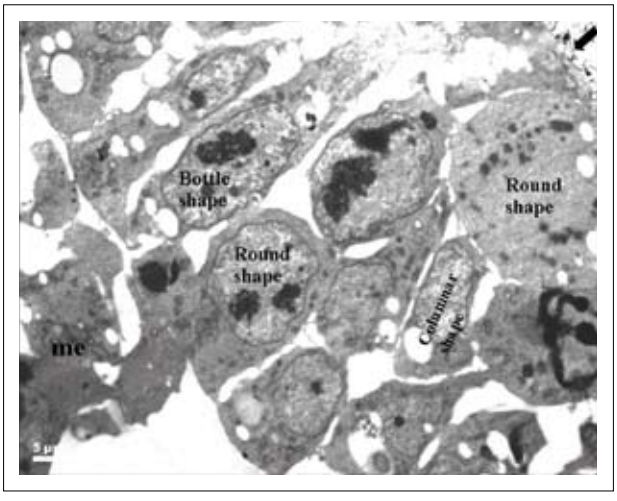

Fig 1. This presents the epiblast layer. This layer arranges as the stratified columnar epithelium which consists about 2-4 layers. The superficial layer consists of columnar and round cells. The subsequent layer is composed of bottle-shaped cells and the lower layer consists of round cells.

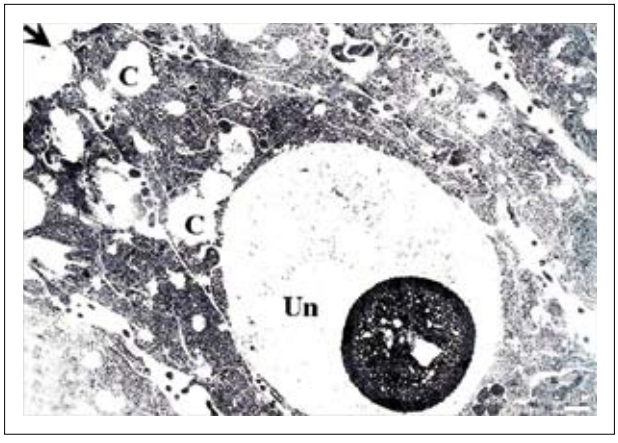

Fig 2. The superficial layer of the stratified columnar epiblast cells, arrow is the apical surface, C is the clear vacuole, un is the unclear vacuole. The lateral side of the cells show several cytoplasmic projections or filopodia to initiate the separation of the cells.

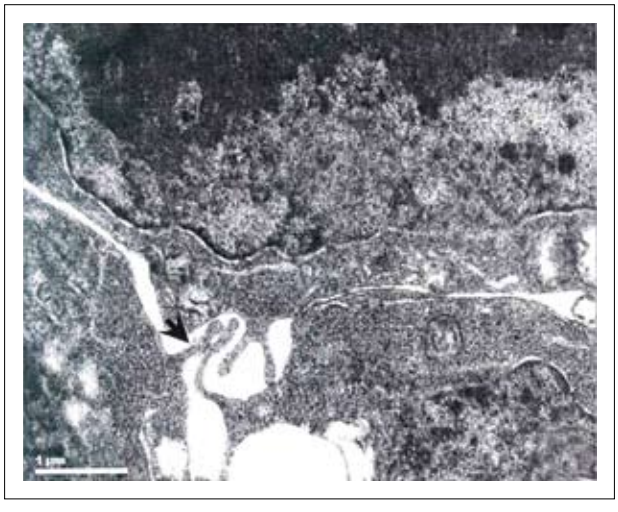

Fig 3. Higher magnification of the filopodia (arrow) at the lateral surface of the epiblast cell, they initiate the separation of the cells, the deeper cell become the mesoblast.

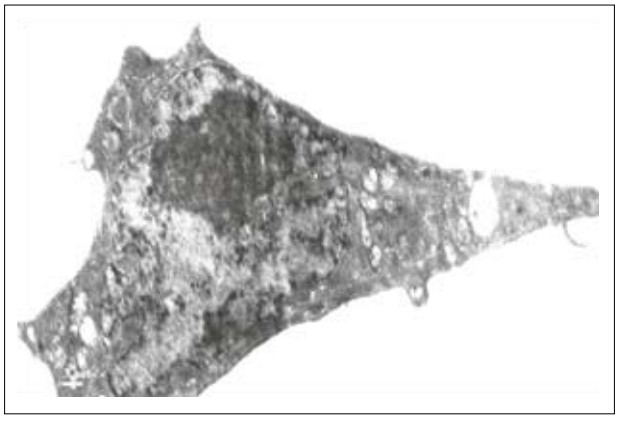

Fig 4. The nucleus of the mesoblast is large an euchromatic with other organelles such as mitochondria, rough endoplasmic reticulum and several vacuole

DISCUSSION

The epiblast layer shows clearly that it is a stratified columnar epithelium and cells proliferate to form more cells for migration and differentiation to be the mesoblast. The bottle-shaped cells locate at the deep layer of the epiblast and prepare themselves for separation by protruding from the filopodia from lateral sides of the cell. Carlson BM[8] described the bottle cells formation with the appearance of the microtubule and microfilament. This cell has a long neck and the enlarged portion is located away from the cells. In this study, the microtubule and microfilament cannot be observed. The nucleus of the epiblast is mostly euchromatic and the nucleolus is very distinct. This cell is therefore highly active in protein synthesis[13] which may be the synthesis of the cytoorganelles. Other cytoorganelles are of embryonic type such as small mitochondria and a short cistern of the rough endoplasmic reticulum. Some epiblast cells have several small mitochondria for energy production which is necessary for cell migration. The vacuoles are very distinct structures derived from a phogocytotic process of the egg white.

The mesoblast cells are derived from migration of the epiblast. Those cells change their shape from columnar to bottle-shaped and are more round with

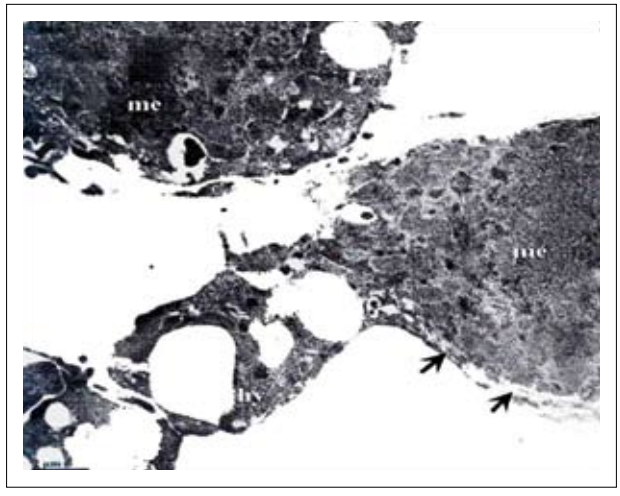

Fig 5. Most mesoblasts migrate between the epiblast and hypoblast and become the mesoderm. Some mesoblasts (me) migrate to the hypoblastic layer (ly) and come closely attached and pressed the hypoblast to be a very thin layer of cytoplasm (arrows).

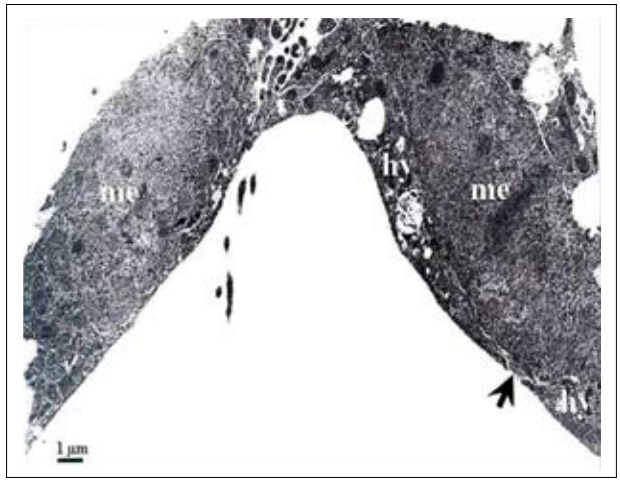

Fig 6. The mesoblasts (me) press the hypoblast (hy) until the hypoblasts separate (arrow) and the replacement occurs.

processes and migrate laterally between the epiblast and hypoblast. Mesoblast cells still exhibit the filopodia which may act for the purpose of movement. The nucleus is euchromatic with one or more distinct nucleoli. The mitochondria are small and more numerous than other organelles.

The hypoblast is a squamous cell with small and flat electrondense nucleus, so there is no proliferative function and also no synthetic activity of cell organelles. The lateral surface contact exhibits filopodia for cell separation. Few organelles can be observed except for the phagocytotic vacuoles.

The epiblast invaginates and migrates to form the mesoblast which is located between the epiblast and hypoblast. Initially, the epiblast cells have to separate from the adjacent cells by protruding from the numerous filopodia from the lateral surface of the cells to loosen the contact. The deeper cells separate from the superficial cells and appear as bottle-shaped cells. The superficial cells still contact with each other by tight junction and become peg-shaped cells. Some epiblast cells migrate ventrally and come to appose with the hypoblast. The hypoblast is becoming a thin and flat form with the very thin sheet of cells, then the cytoplasmic sheet disappears, and gives way for mesoblast. At the later stage of primitive streak, the mesoblast cells increase the number of replacements until the lower layer of the embryonic disc contains mostly mesoblast cells.

CONCLUSION

The origin of the endoderm is from the epiblast of the primitive streak which originally stratified into the squamous epithelium. The cells protrude the filopodia from the lateral surface of each cell to initiate the separation. After cells separate to become the mesoderm, the cells change their shape to round or oval with shorter filopodia which are believed to serve the movement purpose. Some mesoblast cells move ventrally to the hypoblast and depress it as cells contact. The depression force is high enough to make the hypoblast become a thin sheet of cells and eventually disappear. The mesoblast cells then replace the separated area and the number of replacements increases until the hypoblast layer become a simple cuboid instead of squamous. This study so showed clearly the transmission electron microscopic evidence of the origin of the endoderm.

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