Original Article

Early Embryology of the Prechordal Region

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Summary:

Background: *Prechordal plate is important midline structure in the head region. It has been shown that it plays a bivotal role in the development of the brain and the eyes.*

Aim of study*: There is confunsion, in the literatures and textbooks concerning of terms: prechordal plate, prechordal mesoderm and the Buccopharyngeal membrane. The aim of this work is to study is based on histological changes of the early stages of the chick embryo hoping to illuminate these aspects especially after the recent revival of attention to the importance of this region in the process of induction of the brain region of the newral tub & the development of the prosencephalon & the eyes as shown by genes activities & expression in the prechordal plate ..*

Materials & Methods :this study is based on histological description of the early stages (4-7H.&H.) of the chick embryo to illuminate the nature of these structures . *It appeared that the cephalic foregut in the early stages is lined by tall endodermal cells that form three regions: Dorsal wall (P1) where it forms the prechordal plate and contributes to the formation of the prechordal mesodermal mass (mesoendoderm); the rostral end (P2), and the ventral wall (P3) where it fuses with the surface ectoderm forming the oral plate (or the Buccopharyngeal membrane). The contribution of endoderm to the head mesenchyme is emphasized in this study.*

Conclution: *this study indicated that the prechordal plate is not simply the Buccopharyngeal membrare it forms an early proliferating zone , rostal to the notochord , that contribute to the mass of prechordal mesoderm & another zone , which is extending later as a Sessels pouch , which also proliferate for some time . also the ventral extension is a part of Buccopharyngeal membrane.*

Key words: *Chick embryo. Cephalic foregut, Prechordal plate, Prechordal mesoderm, Mesoendoderm, Buccopharyngeal membrane.*

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Introduction

The cephalic axial (mid – line) structures in the early stages of the developing embryo are of special importance, because these act as head- organizer, as described by Speman (1). The notochord is the main axial structure of the body of the embryo, underlying the ectodermal layer that forms the medullary plate. The notochord does not extend to the rostral end of the embryo. There is important axial tissue anterior to the notochord, underlying the medullary plate, that is the prechordal plate, which formed by compact tissue in association with the roof of the foregut. The prochordal (or prochordal) plate is thought to form in later- stage the buccopharyngeal membrane.

In the textbooks (2,3), there is confusions in describing the nature and the origin of the prechordal plate. Some textbooks define it as a localized area of close contact between hypoblast and epiblast (2).

Researchers vary in their considerations between an area of thickened and fused ectoderm and endoderm, and as a condensed mass of mesoderm, giving rise to head mesenchyme in later stages (5,6). Adelman (7,8), who made the most extensive study of the prechordal region in Amblystoma and in chick embryos, had shown that the prechordal region is a pre axial proliferating mesodermal mass. Later workers considered that prechordal mesoderm is formed by cells from epiblast invaginating through Hensens node (6,9,10,11).

These studies contribute enourmasly to our knowledge concerning the origin and development, but did not resolve the existing confusion. The aim of this work is to study the histological changes of the early stages of the chick embryo, hoping to illucidate these aspects, especially after the recent revival of attention to the importance of this region in the process of induction of the brain region of the neural tube and the development of the prosencephalon and

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the eyes as shown by genes activities and expression in the prechordal plate (12,13,14).

Materials and Methods:

Fertilized eggs of the chick (Gallus gallus domesticus) were incubated in 38 C with 70% humidity for periods of 24-48 hours.

At the specified time, the eggs were candled to visualize the embryonic disc. The size of the shadow of the disc gives good indication for the stage of development. A window is then, opened in the shell of the egg at the region of the embryonic disc. The embryos were examined under stereomicroscope to determine the exact stage of development according to Hamburger & Hamilton table (15).

Embryos at stages (4-7) were selected for the study.

Preliminary fixation was performed in- or, by dropping fixative on the embryo by pipette.

The embryo is then removed and properly fixed, either in Bovines (16) or Karnovskys (17) fixatives.

After dehydration the specimens were embedded in paraplast and sectioned serially in 5-micron thickness and stained with Haematoxylene and Eosin. Few specimens were embedded in Araldite, and sectioned using ultracut ultramicrotome into 0.5- 1μ thickness, and stained with Toludine blue.

Results:

Embryo at the specified stages of development represents the development of embryonic disc from the beginning of head fold formation to the beginning of the neural tube formation:

Stage 4 (H. & H.): (Fig.1)

Examination of longitudinal, saggital sections showed that the embryonic disc consists of 3 basic layers. The ectoblastic layer is forming the surface layer. This layer started to show a region of primitive streak, were the cells are loosing their unified shape and become more polymorphic, crowding forming several layers. Some cells are spreading from the mass of primitive streak, between the ectoblast and hypoblast.

The cephalic region shows the beginning of fold formation. The ectoderm in this region is already thicked, forming the medullary (neural) plate. The folding hypoblastic (endodermal) layer is formed at the rostral part by tall cells compared with the rest of hypoblastic layer.

Stage 5 (H. & H.): Histological examination of saggital and cross section of the head region, shows the completion of head fold and formation of the foregut. The notochord process is seen a long the mid line dorsal to the endodermal roof of the gut, but not extending to the rostral part of the gut. Rostrally it merges with a mass of tall cells that line the gut rostrally with several polymorphic cells in association to that layer.

Stage 6 (H. & H.): The head fold is well formed. The saggital and parasaggital sections showed that the cephalic end of the foregut is lined by tall, columnar epithelium. This epithelium forms dorsal wall rostral part, and the ventral cells are in association with more polymorphic cells spreading dorsally in the floor of the medullary plate. Some of these cells are in contact with the neuroepithelium, while in other side they are not separated from the cells that form the roof of the foregut.

Rostrally the thick endodermal lining of the foregut is bending ventrally. In the ventral aspect of the cephalic end of the embryo, the ectoderm layer comes in close contact with the endodermal layer. This ectoderm is also relatively thick, formed by columnar cells and is the continuation of surface ectoderm from the ventral aspect of the anterior neuropore. This ventral part of both endedermal layer and ectodermal layer is what represent the buccopharyngeal membrane.

Stage 7 (H. & H.):

The medullary (neural) plate has formed the neural folds and the beginning of the neural tube formation in the head. The foregut forms an elongated blind sac within the head region. Its roof, anterior and ventral walls appear thick because that the forming cells are tall, columnar and packed.

In the dorsal aspect of the roof, there are more than one layer of cells directly lining the lumen are columnar, but cells dorsal to these are polyhedral or stillate. There is no demarcation (or basement membrane) between the lining cells and other cells. Under higher magnification there are cellular junctions between the cells. But some cells are free and distant from the roof (Fig 4).

Some of the loose cells as well as some polyhedral and tall anchered cells have crystal- like granules in their cytoplasm. Caudal, at the mid line, the notochord is distinguishable by the characteristic features of its cells which are closely packed and homogenous cytoplasm, at this stage.

The proliferating thick roof of the foregut extends anteriorly, but in the ventral aspect of the gut, the wall is thick and formed by densely packed tall cells, mostly forming a single layer. The surface ectodermal cells in this region form points of contact with the gut endoderm.

Discussion:

The application of recent advances in genomics and molecular biology, in the study of embryonic development, revived the attention on the prechordal plate as an inductive source for the development of the forebrain, eyes, and the head in general (12). The cells of this region express the Sonic Hedgehog gene (SHH) and Bon Maturation Protein-7 (BMP7) and the goosecoid factors (13). The prechordal plate and

mesoderm, were described in vertebrates embryos long ago. But controversies concerning the nature and origin of this plate and the mesoderm remained unsolved. Different openions in defining the plate and its fate. The prevailing view is swinging between the view that considers the plate as a thickening and fusion of both endoderm and ectoderm to form the buccopharyngeal plate (3).

On other hand there is the prechordal mesoderm which is mostly formed by migrating cells from epiblast invaginating through Hensens node (6,10) and perhaps with contribution from neural crest on later stages (18).

In this study of early stages of the chick embryo, which still considered as a model for higher vertebrate embryology, the prechordal plate appears early at stage (4) at the beginning of head fold formation, as rostal area of endodermal epithelium which is made by tall cells while, the endoderm in other parts is formed by flattened or cuboidal cells.

This epithelium is forming the roof and extending to the rostral end and ventrally of the forming foregut. This might be the same region of the hypoblast present in the cephalic margin of the embryonic disc of earlier stages.

During stages 5and 6 (H. & H.) there was obvious compaction of the cells of this rostral part of the gut. The thickened endoderm presents three parts: The roof (p1), the rostral end (P2) and the rostral ventral wall (P3) (Fig 5). "P1" could be seen to represent a proliferating zone giving rise to most of the prechordal mesoderm with contribution from invaginating and migrating cells. This mass is formed by several layers of cells in continuity with the lining the gut dorsally. No demarcation could be seen between different layers of this mass. A basement membrane was described to appear in later stages between the lining epithelium and the rest of the mss (5). The contribution of the endoderm to this mass agrees with the term " mesoendoderm"mentioned by Knoe'gen et al (19) and by Rubestein et al (20) from endoderm to mesenchymal tissue is not commonly seen, but personal observation on other sites like the endodermal pharyngeal pouches indicate such possibility.

"P2" is another site of proliferating endodermal region, but in latter stages when forming a dorsal extension from the rostal end of the gut toward the base of diencephalon that is forming the Sessels pouch which might contribute cells into the developing pituitary gland (Hamash et al (12)). P3-part, which is also athicked endoderm, will come in close contact with surface ectoderm, to form together the oral plate or the Buccopharyngeal membrane.

In this region there will be an extensive process of apoptosis and loosening of cells resulting in disintegration of the membrane.

In conclusion, this study indicated that the prechordal plate is not simply the Buccopharyngeal membrane. It forms an early proliferating zone, rostral to the notochord, that contribute to the mass of prechordal mesoderm and another zone, which is extending later as a Sessels pouch, which also proliferate for some time. Thirdly the ventral extension is a part of Buccopharyngeal membrane.

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Figure 1: A photomicrograph of stage 4 (H. & H.) of chick embryo – cephalic part, showing the beginning of head folding. Tall epithelial cells characterize cephalic part of Endoderm. Ec – Ectoderm; En – Endoderm. (X 240)

Figure 2: A photomicrograph of sagittal section at head region of chick embryo at stage 5 (H. & H.) showing the cephalic of the foregut. The dorsal, rostral and ventral walls are lined by tall, columnar epithelium of endoderm. (X 360)

Figure 3 : A photomicrograph of parasagittal section of head region of chick embryo at stage 7 (H.& H.). The dorsal wall of cephalic foregut is lined by tall cells in association of mesenchymal cells more dorsally, that form the prechordal mesoderm. (X 240)

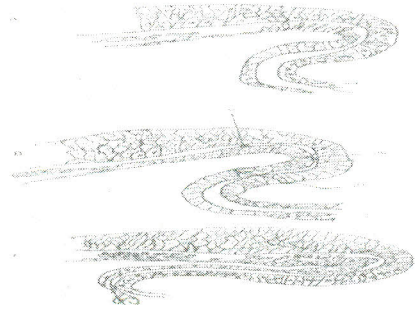

Figure 4 : Diagrammatic drawings of various stages in development of cephalic part of foregut .

A – Represent stage 4 – 5 (H.& H.). The head fold is completed . The cephalic foregut is lined by tall , columnar endodermal epithelium .

B – Represent stage 5 –6 (H.& H.) .There is obvious pooling of tall cells forming the dorsal (P1), rostral (P2) and ventral (P3) walls of foregut .

C – Represent stage 7 (H. & H.). The dorsal walls epithelium (P1) is giving rise to mesenchymal cells that contribute & prechordal mass of mesoderm .

(P2) is the ventral wall of foregut, which is fusing with surface ectoderm to form buccopharyngeal membrane

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