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Research Article

Ventral neural tube cells differentiate into hepatocytes in the chick embryo

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Abstract. A population of ventral neural tube cells has recently been shown to migrate out of the hindbrain neural tube via the vagus nerve and contribute to the developing gastrointestinal tract. Since liver is also innervated by the vagus nerve, we sought to determine if these cells also migrate into the liver. Ventral neural tube cells in the caudal hindbrain of chick embryos were tagged with a replication-deficient retroviral vector containing the LacZ gene on embryonic day 2. Embryos were processed for detection of labeled cells on embryonic day 5 and 11. Labeled cells were seen in the liver on both days and identified as hepatocytes. Previously, it was believed that all hepatocytes develop from the gut endoderm. Results of the present study show an additional source for the formation of liver cells.

Key words. Ventral neural tube cells; chick embryo; hepatocytes; development; liver.

Neural crest cells detach from the dorsal portion of the developing neural tube and contribute to the formation of a wide variety of structures [1, 2]. It was previously believed that after the emigration of neural crest the remaining neural tube cells give rise to only the neurons and supporting cells of the central nervous system. In other words, only neural crest cells emigrated from the neural tube. Results of several studies have now indicated that some neural tube cells also emigrate and contribute to the formation of structures developing outside the central nervous system. For example, in the case of the spinal cord, dorsal neural tube cells as well as ventral neural tube cells emigrate via the dorsal and ventral roots in avian embryos [3–6]. The emigrated neural tube cells differentiate into neural and nonneural cell types [3–6]. Emigration of neural tube cells is not limited to the spinal cord, as it also occurs in the cranial portion of the neural tube. Studies utilizing focal application of the vital dye DiI, homeobox gene Islet-1 expression pattern and retroviral labeling have shown that some cells originate in the ventral portion of the hindbrain neural tube and migrate out at the site of attachment of cranial nerves in avian embryos [7–9]. Emigration of ventral neural tube cells begins considerably after the time of completion of emigration of neural crest [7–9]. The emigrated ventral neural tube cells differ from neural crest as they do not express the neural crest cell marker HNK-1 [8, 9]. Thus, the neural tube provides two cell populations, the neural crest and the neural tube cells, for the formation of structures developing outside the central nervous system.

A recent study reported migration of ventral neural tube cells into the developing gastrointestinal tract in chick

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embryos [8]. Ventral neural tube cells in the hindbrain were labeled with replication-deficient retroviral vectors containing the LacZ gene. This method permanently labels neural tube cells and thus permits observations on the fate of the emigrated cells [10]. The vector was introduced into the lumen of the neural tube on embryonic day 2. The labeled cells on day 3 were restricted to the inside of the neural tube. On day 4, labeled cells emigrated in close association with the vagus nerve and migrated into the developing gastrointestinal tract. They differentiated into the smooth muscle cells of the intestine and stomach [8]. Since the ventral neural tube cells migrate in association of the vagus nerve, which also goes to the liver, we sought to determine if these cells also migrated into the developing liver.

Materials and methods

Fertilized Arbor Acre chicken eggs were incubated at 37.5 °C. During the second day of incubation, an opening in the egg shell was made to acquire access to the embryo. Replication-deficient retroviral vector LZ14, provided by Deni S. Galileo [11, 12], Department of Cellular Biology and Anatomy, Medical College of Georgia, was introduced into chick embryos at stage 14 of Hamburger and Hamilton [13], as described in detail previously [8]. Briefly, approximately 0.2 μl of the viral concentrate was injected into the lumen of the caudal hindbrain neural tube using a glass micropipet connected to a pneumatic pico pump under a dissecting microscope. In control embryos, the virus was injected outside the hindbrain neural tube. The placement of the virus outside the neural tube did not label cells in the embryo, as also was reported previously [8, 9].

Embryos were fixed in 2% formaldehyde in phosphate-buffered saline (PBS) on embryonic days 5 and 11. They were processed for histochemical detection of LacZ with the substrate Bluo-gal (Sigma) as described previously for X-gal [11, 12]. Tissues were postfixed in the same fixative and cleared in 70% glycerol. The cleared embryos were placed in PBS overnight, dehydrated and embedded in paraffin. They were sectioned at 5 or 15 μm thickness and stained with eosin or hematoxylin and eosin. Sixty-one embryos were processed, and 11 embryos showed labeled cells in the liver.

To insure that the tagged cells were hepatocytes, a specific marker was used. The presence of intracellular albumin was used to identify hepatocytes positively. The immunocytochemical procedure was the same as described in detail previously [8]. Briefly, sections were rinsed and blocked with 20% goat serum in PBS for 1 h, followed by incubation with polyclonal antialbumin (Dako) diluted 1:20 in 5% goat serum for 2 h at room temperature. After rinsing, sections were incubated with horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G (IgG) (1:100, Southern Biotechnology, Birmingham, AL, USA) in 5% goat serum followed by development in diaminobenzidine (Sigma). Preimmune rabbit serum was used as control.

Results

In the chick embryo, the liver primordium becomes visible as an endodermal evagination of the foregut on embryonic day 3 [14]. By embryonic day 5, hepatic trabeculae containing differentiated hepatocytes can be easily distinguished histologically from the sinusoids [14]. A histological section through the liver of an embryonic day 5 embryo is shown in figure 1A. The labeled cells are located in the trabeculae and are not seen in the sinusoids. Some labeled cells appear to be hepatocytes, based on their location and size.

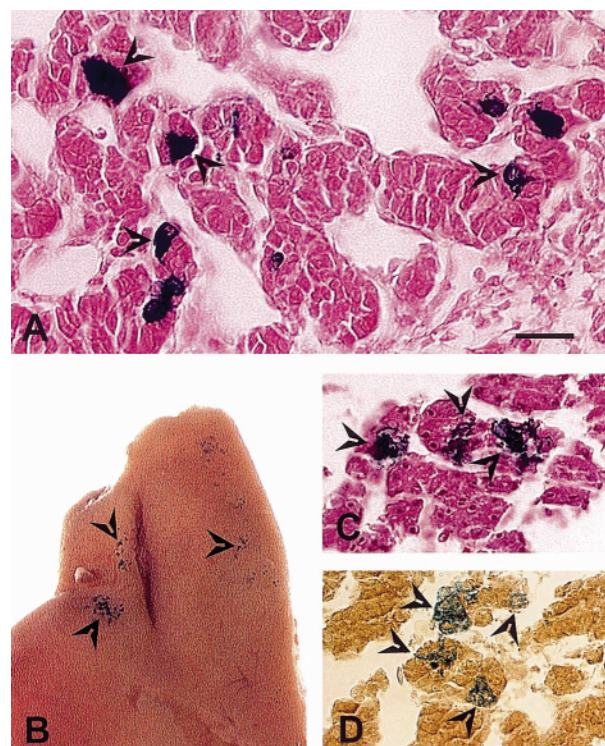

Figure 1. Ventral neural tube cells differentiate into hepatocytes in the chick embryo. (*A*) Histological section, stained with eosin (without hematoxylin), through the liver on embryonic day 5 showing retrovirally labeled cells (arrowheads). (*B*) Whole mount of an embryonic day 11 liver showing clusters of labeled cells (arrowheads). (*C*) Section, stained with hematoxylin and eosin, through an embryonic day 11 liver showing retrovirally labeled cells (arrowheads). (*D*) Section through an embryonic day 11 liver immunostained with antibody to albumin showing the retrovirally labeled cells stained positively (arrowheads) for the hepatocyte marker. Bar, 5 μm (*A*), 300 μm (*B*), 20 μm (*C*) and 30 μm (*D*).

A whole mount of part of an embryonic day 11 liver is shown in figure 1B. Clusters of labeled cells can be seen in several distinct locations. A histological section of an 11-day liver is shown in figure 1C. The size and location of the labeled cells suggested that they may be hepatocytes. To confirm the identity of the labeled cells, sections were stained with antibody to albumin, a specific marker for hepatocytes. Hepatocytes synthesize albumin, so are labeled intracellularly. Cells in other organs normally exclude albumin. Figure 1D shows that the retrovirally labeled cells stained positively with the marker, indicating that they were hepatocytes.

Discussion

The results of the present study show that retroviral labeling of the ventral neural tube cells in the caudal hindbrain of chick embryos later results in the presence of labeled cells in the liver. The labeled cells were identified as hepatocytes.

It is generally believed that the liver develops from two sources of cells: endoderm and mesoderm [15]. The endoderm is believed to give rise to all hepatocytes. The other principal types, that is endothelial, Kupffer and Ito cells, are believed to be derived from mesoderm. Results of the present study indicate a third source of cells for the developing liver, the ventral neural tube cells of the hindbrain.

Neural crest cells originate from the dorsal portion of the developing neural tube and contribute to the gastrointestinal tract by seeding the enteric nervous system [1]. The possibility that neural crest cells were labeled in the present study is considered unlikely for the following reasons. Neural crest cells from the cranial neural tube complete their emigration by about stage 11 in the chick embryo [16–18]. The ability of the neural tube to generate new neural crest is also lost by stage 11 [19]. In the present study, the virus was injected at stage 14, that is considerably after the completion of emigration of neural crest. Further, we have shown previously that the ventral neural tube cells associated with the vagus nerve and the gastrointestinal tract do not express the neural crest marker HNK-1, and some of the embryos utilized in this study were part of the same series [8].

The possibility that the labeling represents artifacts is also considered unlikely for several reasons. The vector employed is replication-deficient. Batches were routinely tested as described previously [11, 12], and they were found to be free of the wild-type virus. Further, injection of the virus outside the neural tube does not result in labeling of cells in the embryo [8, 9]. Although the liver is a hematopoietic organ, the formation of blood cells in the liver does not occur until the seventh and eighth day of incubation [14]. In the present study, labeled hepatocytes were observed in embryonic day 5 embryos. Thus, it is unlikely that the observed results represent artifactual labeling.

The most likely explanation for our results is that an additional source of cells participates in the differentiation of cells in the liver. The precise functional significance of this novel source of cells is yet to be determined.

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