Aberrant Differentiation of the Axially Condensed Tail Bud Mesenchyme in Human Embryos With Lumbosacral Myeloschisis

HIROTOMO SAITSU,[1]* SHIGEHITO YAMADA,[2] CHIGAKO UWABE,[2] MAKOTO ISHIBASHI,[1] AND KOHEI SHIOTA[1,2]

[1]Department of Anatomy and Developmental Biology, Graduate School of Medicine, Kyoto University, Kyoto, Japan

[2]Congenital Anomaly Research Center, Graduate School of Medicine, Kyoto University, Kyoto, Japan

ABSTRACT

Development of the posterior neural tube (PNT) in human embryos is a complicated process that involves both primary and secondary neurulation. Recently, we histologically examined 20 human embryos around the stage of posterior neuropore closure and found that the axially condensed mesenchyme (AM) intervened between the neural plate/tube and the notochord in the junctional region of the primary and secondary neural tubes. The AM appeared to be incorporated into the most ventral part of the primary neural tube, and no cavity was observed in the AM. In this study, we report three cases of human embryos with myeloschisis in which the open primary neural tube and the closed secondary neural tube overlap dorsoventrally. In all three cases, part of the closed neural tube was located ventrally to the open neural tube in the lumbosacral region. The open and closed neural tubes appeared to be part of the primary and the AM-derived secondary neural tubes, respectively. Thus, these findings suggest that, in those embryos with myeloschisis, the AM may not be incorporated into the ventral part of the primary neural tube but aberrantly differentiate into the secondary neural tube containing cavities, leading to dorsoventral overlapping of the primary and secondary neural tubes. The aberrant differentiation of the AM in embryos with lumbosacral myeloschisis suggests that the AM plays some roles in normal as well as abnormal development of the human posterior neural tube. Anat Rec, 290:251–258, 2007.

Key words: axially condensed mesenchyme; myeloschisis; human embryo; primary neural tube; secondary neural tube

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Hirotomo Saitsu's present address is Department of Human Genetics, Graduate School of Medicine, Yokohama City University, Yokohama, Japan.

*Correspondence to: Hirotomo Saitsu, Department of Human Genetics, Graduate School of Medicine, Yokohama City University, Yokohama 236-0004, Japan. Fax: 81-45-786-5219.
E-mail: hsaitsu@yokohama-cu.ac.jp

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In human embryos, the neural tube forms by means of two distinct developmental events, i.e., primary and secondary neurulation. During primary neurulation, the lateral ends of the neural plate elevate and bilateral neural folds fuse with each other to form the primary neural tube. Primary neurulation is completed by the closure of the anterior and posterior neuropores, at 24 and 28 days, respectively, after fertilization (O'Rahilly and Müller, 1994). Subsequently to the closure of the posterior neuropore (the last part of the primary neural tube to be fused), the secondary neural tube begins to develop by elongation and cavitation of the tail bud, an aggregate of undifferentiated mesodermal cells at the caudal end of embryos. This process is called secondary neurulation (Griffith et al., 1992; O'Rahilly and Müller, 1994; Colas and Schoenwolf, 2001). Closure of the posterior neuropore occurs at the upper sacral level during Carnegie stage 12 (CS 12) (Müller and O'Rahilly, 1987; Nakatsu et al., 2000). Although the closure of the posterior neuropore occurs at the level of the thirty-first somite (corresponding to the future S2 level) (Müller and O'Rahilly, 1987; Nakatsu et al., 2000; O'Rahilly and Müller, 2003), the junction of the primary and secondary neural tubes is apposed at the lumbosacral level of the vertebral column in neonates (O'Rahilly and Müller, 2003). Therefore, development of the posterior neural tube (PNT), which develops into the future lumbar, sacral, coccygeal, and equinal cord, involves both the primary and secondary neurulation and is a rather complicated process.

Failure in primary and secondary neurulation results in various forms of neural tube defects (NTD), which are among the most common human congenital malformations, affecting 0.5–8/1,000 live births (Little and Elwood, 1992). Nonclosure of the anterior and posterior neuropores can result in exencephaly/anencephaly and myeloschisis, respectively. Myeloschisis occurs most frequently at the lumbosacral level of the vertebral column (Dryden, 1980), which corresponds to the junctional region between the primary and secondary neural tubes in neonates (O'Rahilly and Müller, 2003). Therefore, PNT development in the junctional region should be examined carefully in order to understand the pathogenesis of myeloschisis. Recently, we histologically examined 20 human embryos around the stage of posterior neuropore closure (Saitsu et al., 2004). Notably, the axially condensed mesenchyme (AM) intervened between the neural plate/tube and the notochord at the stage of posterior neuropore closure in the junctional region between the primary and secondary neural tubes, while the notochord was directly attached to the neural plate/tube in the rostral region. The AM located between the primary neural tube and notochord appeared to be incorporated into the most ventral part of the primary neural tube, and no cavity was observed contrary to the chick medullary cord. After the posterior neuropore closed, early cavitation of the secondary neural tube was found to start at the caudal end of the primary neural tube with radial rearrangement of AM cells. Thus, it seems that the AM plays important roles in development of the PNT in the junctional region between the primary and secondary neural tubes. Because myeloschisis occurs most frequently in the junctional region, we hypothesized that failure of normal development of AM plays some roles in the development of human myeloschisis. In a large collection of human embryos (the Kyoto Collection of Human Embryos, Kyoto University), we encountered three embryo cases with lumbosacral myeloschisis in which the open primary neural tube and the closed secondary neural tube overlapped dorsoventrally. In all three cases, part of the closed secondary neural tube was located ventrally to the open primary neural tube in the lumbosacral region. Thus, it seemed that the AM failed to be incorporated into the ventral part of the primary neural tube and aberrantly differentiated into secondary neural tube, resulting in dorsoventral overlapping of primary and secondary neural tubes. Such abnormal differentiation of the AM in embryos with lumbosacral myeloschisis suggests that the AM plays some roles in normal and abnormal development of the human posterior neural tube in the junctional region.

MATERIALS AND METHODS

The human embryos examined in this study were from the Kyoto Collection of Human Embryos held in the Congenital Anomaly Research Center of Kyoto University. The embryo collection consists of approximately 44,000 embryos, most of which were procured after termination of pregnancy given to healthy women for social reasons (Maternity Protection Law of Japan). Most embryos in the collection were within 8 weeks after fertilization. Further details of the embryo collection and its demographic characteristics have been previously described elsewhere (Nishimura, 1975; Matsunaga and Shiota, 1977; Shiota, 1991). The embryos were fixed in 10% formalin or Bouin fluid soon after procurement, and after being sent to the laboratory in Kyoto University, they were staged (O'Rahilly and Müller, 1987), measured, and examined for structural abnormalities and signs of intrauterine death under a dissection microscope. Some of the well-preserved embryos were photographed and serially sectioned at 10 μm thickness for histological examination. In the present study, we examined three embryos at Carnegie stages (CS) 16, 18, and 20 with dorsoventral overlapping of the open and closed neural tubes associated with lumbosacral myeloschisis. They were examined both macroscopically and microscopically. All the embryos except for one (no. 18289) had no sign of intrauterine death. We included the embryo (no. 18289) in this study because it was well preserved. Embryo no. 18973 was associated with holoprosencephaly. We used free software DeltaViewer (http://vivaldi.ics.nara-wu.ac.jp/~wada/DeltaViewer/index.html) for 3D reconstruction. The detailed procedure for reconstructing 3D images from serial sections will be described elsewhere (Yamada et al., 2007).

RESULTS

In the previous study, we found that the axially condensed mesenchyme intervened between the neural plate/tube and the notochord in the junctional region of the primary and secondary neural tubes around the stage of posterior neuropore closure (Fig. 1) (Saitsu et al., 2004). The AM appeared to be incorporated into the most ventral part of the primary neural tube, and no cavity was observed in the AM contrary to the chick medullary cord (Fig. 1).

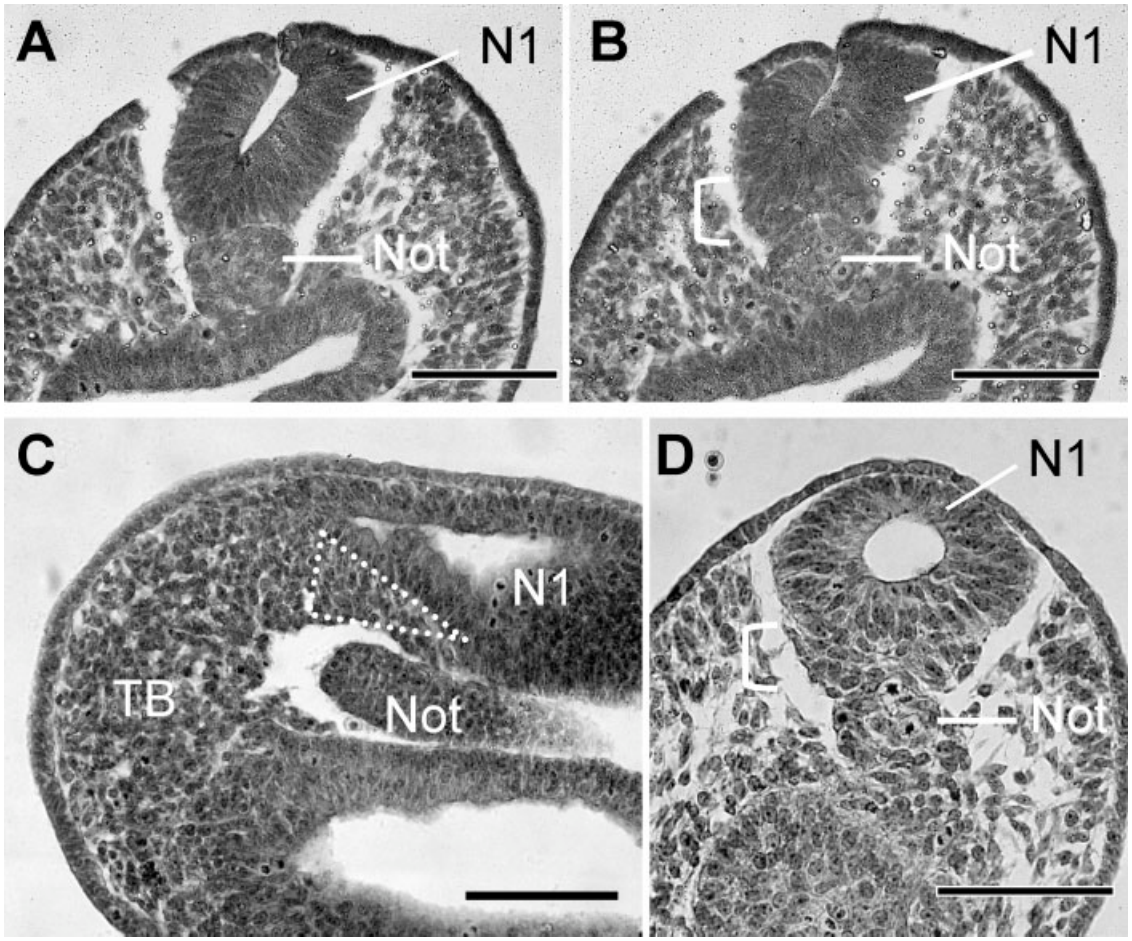

Fig. 1. **A** and **B:** Transverse sections of a CS 12 embryo (No.589) at approximately 20 μm intervals through the closing posterior neuropore in a rostrocaudal sequence. The AM intervenes between the notochord (Not) and the primary neural tube (N1; B, bracket), although the notochord is closely attached to the primary neural tube in more rostral sections (A). **C** and **D:** Sections of the junctional region of the primary and secondary neural tubes in late CS 12 embryos. C: Sagittal section of no. 13087 embryo. The notochord is attached to the primary neural tube almost along its entire length. As the primary neural tube tapers caudally, the AM intervenes between the notochord and the primary neural tube (white broken box). TB, tail bud. D: Transverse section of no. 3005 embryo. The AM intervenes between the notochord and the primary neural tube (bracket). The AM appeared to be incorporated into the most ventral part of the primary neural tube (B–D). Scale bar = 100 μm.

In the present study, we found that three embryos with lumbosacral myeloschisis showed dorsoventral overlapping of the open and closed neural tubes. It seemed that the AM abberantly differentiated into the secondary neural tube. As a consequence, most likely the primary and secondary neural tubes were separated in this region. In the first case of CS 16 embryo (no. 7728, Fig. 2), the open neural tube was observed caudal to the level of the hind limb and the exposed neural tissue appeared overgrown (Fig. 2A, white arrows). To investigate the anatomical relationship between the open primary and closed secondary neural tubes in three dimensions, we reconstructed 3D images of the neural tubes in the vicinity of the caudal end of the open neural tube (Fig. 2C–E). In the dorsal view, the secondary neural tube was found to extend caudally to the open primary neural tube (Fig. 2C). Furthermore, in the ventral and lateral views, there was a dorsoventral overlapping between the open primary and closed secondary neural tubes (Fig. 2D and E, bracket). This feature was also confirmed in serial sections (Fig. 2H–K). It was noted that the closed secondary neural tube was intercalated between the open neural tube and the notochord (Fig. 2H–K), suggesting that the closed secondary neural tube may be derived from AM intervening between the neural plate/tube and the notochord (Saitsu et al., 2004).

In the second case with holoplosencephaly (no. 18973, CS 18; Fig. 3), the open neural tube was also observed caudal to the level of the hind limb (Fig. 3A, white arrows). In the lateral and dorsal views of the reconstructed 3D image of the neural tubes, the secondary neural tube extends caudally from the end of the open primary neural tube (Fig. 3C and E). In addition, in the ventral view, the closed secondary neural tube was found to overlap partially with the open primary neural tube (Fig. 3D, bracket). In transverse serial sections, it

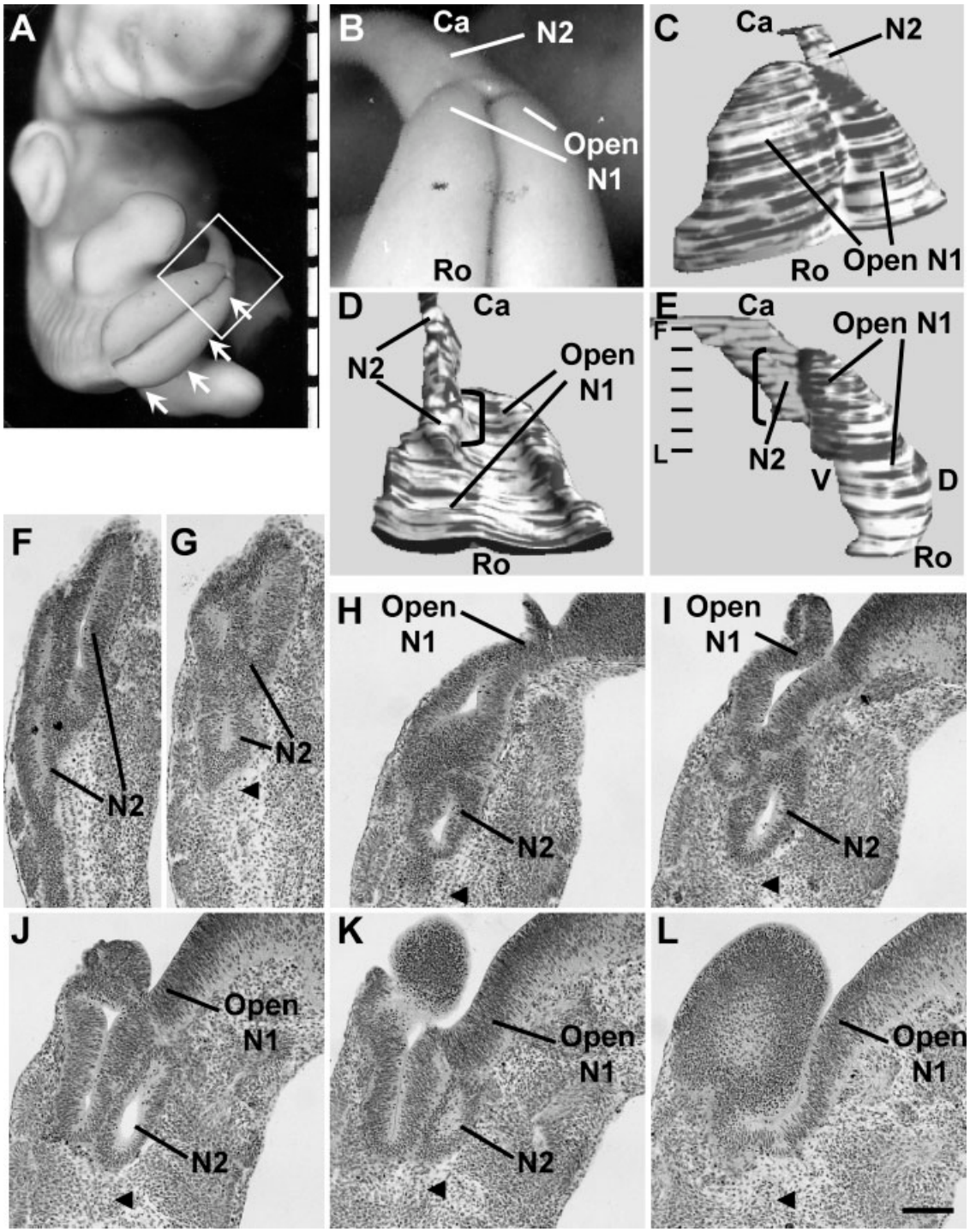

Fig. 2. A CS 16 embryo with lumbosacral myeloschisis (no.7728). **A:** A frontal view of the embryo. The open neural tube is observed caudal to the level of the hind limb (white arrows). **B:** A close-up dorsal view of the myeloschisis region indicated by a white box in A. **C:** The neural tubes in this region are 3D-constructed (compare C with B). In the dorsal view (C), the secondary neural tube (N2) extends caudally from the open primary neural tube (open N1). When this 3D image was observed from ventral (**D**) and lateral (**E**) side, it is evident that there is a dorsoventral overlapping between the open primary and closed secondary neural tubes (bracket). D, dorsal; V, ventral; Ro, rostral; Ca, caudal. **F–L:** Serial transverse sections at the levels indicated by bars in E. Dorsal is top. The closed secondary neural tube is observed beneath the open primary neural tube (H–K). Note that the closed secondary neural tube is located between the open neural tube and the notochord (H–K). The notochord is indicated by a black arrowhead (G–L). Scale bar = 100 μm (F–L).

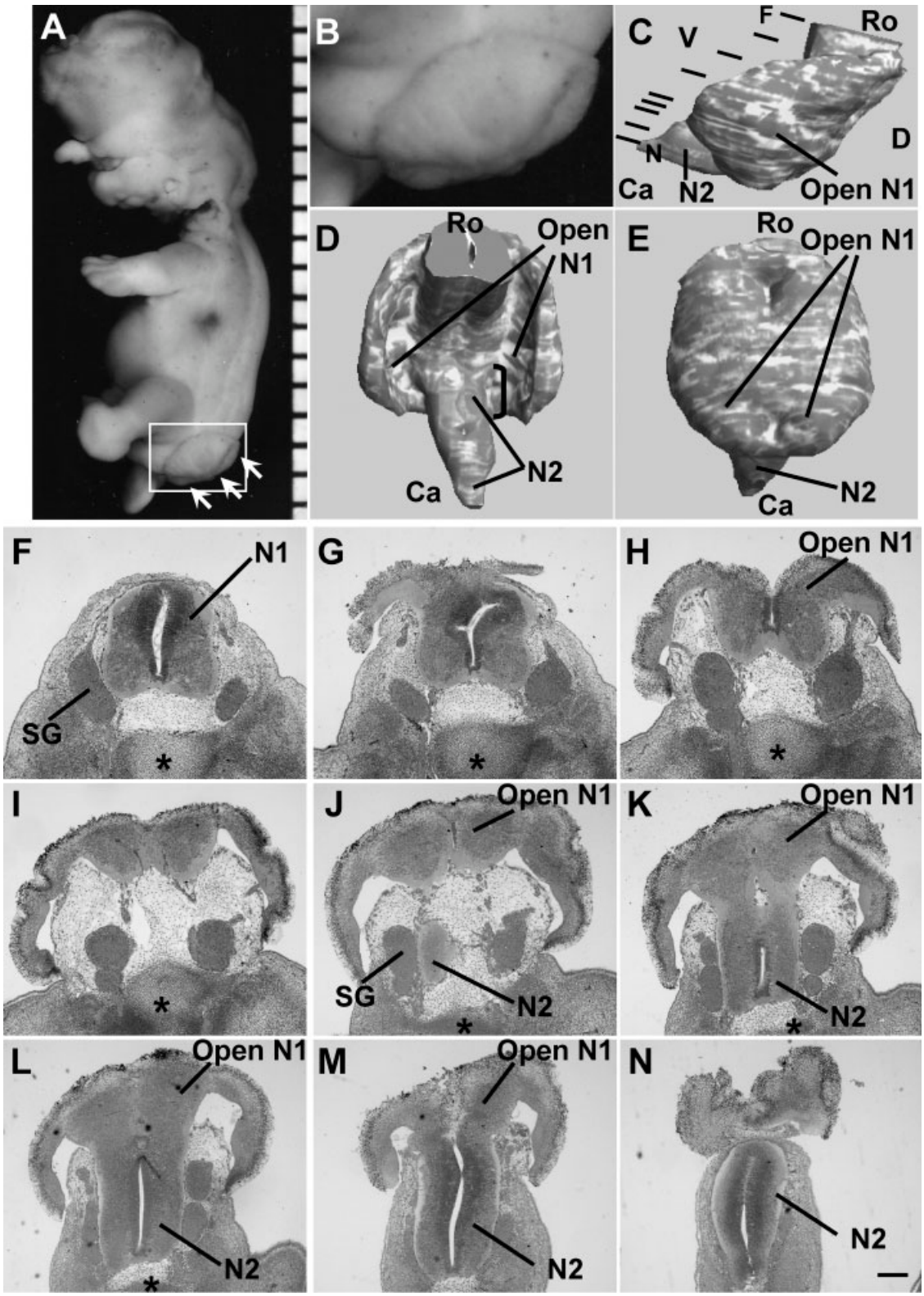

Fig. 3. A CS 18 embryo with lumbosacral myeloschisis (no. 18973). The embryo also shows holoprosencephaly. **A:** A lateral view of the embryo. The open neural tube is observed caudal to the level of the hind limb (white arrows). **B:** A close-up lateral view of the myeloschisis region indicated by a white box in (A). **C:** The neural tubes in this region are 3D-constructed (compare C with B). In the lateral (C) and dorsal (**E**) views, the secondary neural tube (N2) extends caudally from the open primary neural tube (open N1). In addition, in the ventral view (**D**), a dorsoventral overlapping between the open primary and closed secondary neural tubes is clearly observed (bracket). D, dorsal; V, ventral; Ro, rostral; Ca, caudal. **F–N:** Serial transverse sections at the levels indicated by bars in C. Dorsal is top. The closed secondary neural tube is located between the open neural tube and the vertebral body (asterisk; J–M). SG, spinal ganglion. Scale bar = 200 μm (F–N).

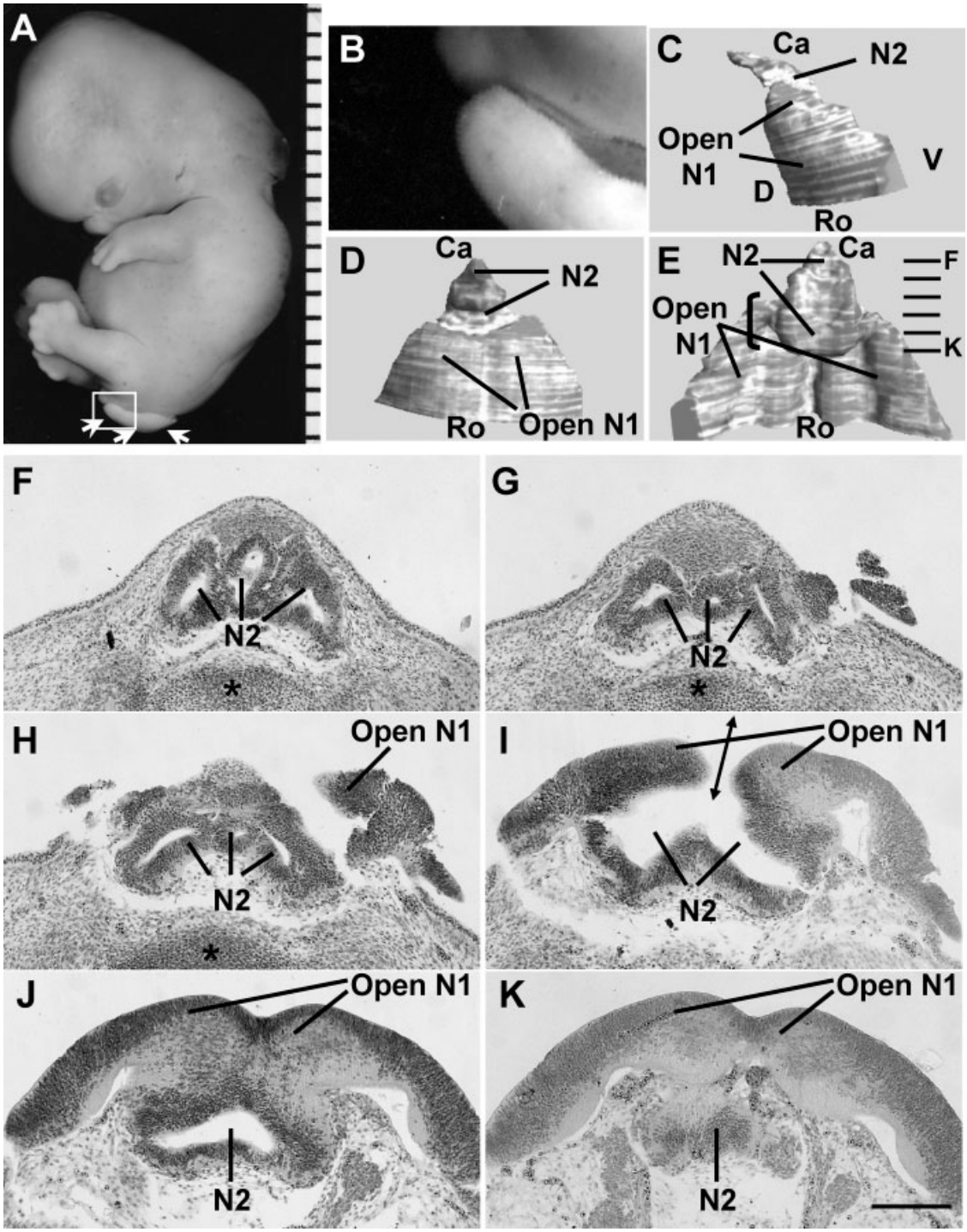

Fig. 4. A CS 20 embryo with lumbosacral myeloschisis (no. 18289). **A:** A lateral view of the embryo. The open neural tube is observed caudal to the level of the hind limb (white arrows). **B:** A close-up lateral view of the myeloschisis region indicated by a white box in A. **C:** The neural tubes in this region are 3D-constructed (compare C with B). In the lateral (C) and dorsal (**D**) views, the secondary neural tube (N2) extends caudally from the open primary neural tube (open N1). In addition, in the ventral view (**E**), a dorsoventral overlapping between the open primary and closed secondary neural tubes is clearly observed (bracket). D, dorsal; V, ventral; Ro, rostral; Ca, caudal. **F–K:** Serial transverse sections at the levels indicated by bars in E. Dorsal is top. The lumens of the secondary neural tube only partly connect to the open lumen of the primary neural tube (double-ended arrow in I) and become separated from the primary neural tube to form a distinct lumen ventral to the open primary neural tube (J and K). The secondary neural tube shows multiple cavities (F–H), which is consistent with our previous report (Saitsu et al., 2004). The vertebral body is indicated by asterisk (F–H). Scale bar = 200 μm (F–K).

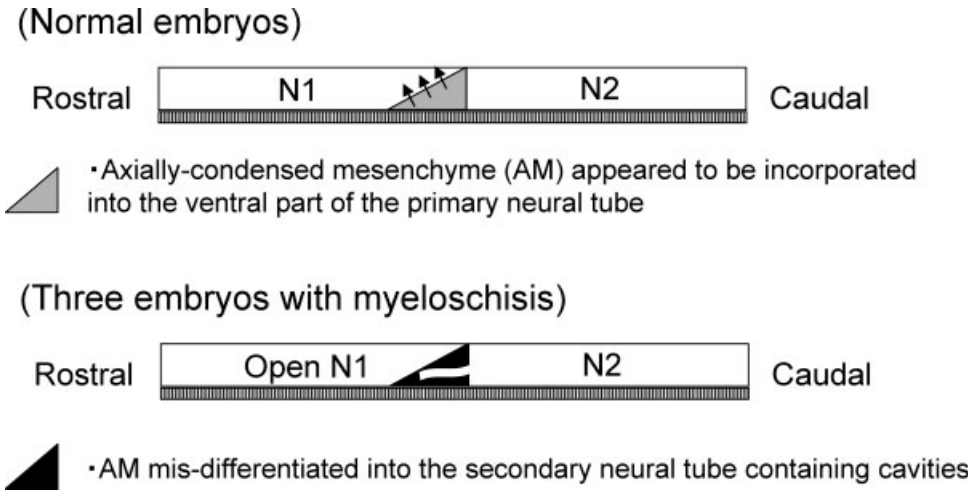

Fig. 5. Summary scheme of axially condensed mesenchyme development in normal embryos and those with myeloschisis. In normal embryos, the AM in the junctional region of the primary and secondary neural tubes (N1 and N2, respectively) appeared to be incorporated into the ventral part of the primary neural tube. On the other hand, the AM appeared to differentiate aberrantly to form the secondary neural tube containing cavities in the three embryos with myeloschisis.

was observed that part of the closed secondary neural tube was located ventrally to the open primary neural tube at their junction (Fig. 3J–N).

In the third case (no. 18289, CS 20), the open neural tube was also observed caudal to the level of the hind limb (Fig. 4A, white arrows). In a ventral view of the 3D image of the neural tubes, the closed secondary and open primary neural tubes partially overlapped at their junction (Fig. 4E, bracket). As illustrated on the caudal–rostral series of sections (Fig. 4F–K), the lumens of the secondary neural tube only partly connected to the open lumen of the primary neural tube (Fig. 4I, double-ended arrow) and became separated rostrally from the primary neural tube to form a distinct lumen ventral to the open primary neural tube (Fig. 4J and K).

These features suggest that in the three cases with lumbosacral myeloschisis, the secondary neural tube abnormally connected to the primary neural tube. In both the second and third cases (no. 18973 and 18289; Figs. 3 and 4), the secondary neural tube was located between the open neural tube and the developing vertebral body, which surrounded the notochord (Fig. 3J–L). It was noteworthy that the overlapping of the secondary and primary neural tubes was constantly aligned along the dorsoventral axis, i.e., the secondary neural tube was located ventrally to the primary neural tube. These findings suggest that the closed secondary neural tube may be derived from AM (Saitsu et al., 2004). In the first and third cases (no. 7728 and 18289; Figs. 2 and 4), the secondary neural tube had multiple cavities (Figs. 2F and G and 4F–H), which is consistent with our previous report (Saitsu et al., 2004).

DISCUSSION

The difference in the AM between normal embryos and three embryos with lumbosacral myeloschisis is summarized in Figure 5. In the present study, we report three cases of human embryos with dorsoventral overlapping of open and closed neural tubes associated with myeloschisis. The overlapping secondary neural tube appeared to be derived from the AM intervened between the neural plate/tube and the notochord around the stage of posterior neuropore closure (Saitsu et al., 2004). Because the AM appeared to be incorporated into ventral part of the primary neural tube in embryos with normal appearance (Fig. 1) (Saitsu et al., 2004), we thought that the AM may aberrantly differentiate into secondary neural tube in three cases. Considering the common occurrence of the myeloschisis in the lumbosacral region, the juctional region of the primary and secondary neural tube, the existence of abnormal development of the AM in the junctional region is an interesting feature. There is one possibility that abnormal development of the other tissues such as neuroepithlium may affect the differentiation of the AM. On the other hand, coexistence of the abnormal differentiation of the AM in three cases raises the possibility that the AM plays some roles in the development of human myeloschisis. It is interesting to note that curly tail embryos showed a reduced rate of cell proliferation in the hindgut endoderm and notochord in the posterior neuropore (PNP) region (i.e., junctional region between primary and secondary neural tubes), resulting in a cell proliferation imbalance in the caudal region of curly tail embryos, with slower growth specifically in ventral midline tissues (van Straaten and Copp, 2001). It is believed that a dorsoventral cell proliferation imbalance leads to temporarily increased ventral curvature of the PNP region and causes a delay of closure of the PNP (van Straaten and Copp, 2001). Therefore, it would be possible that abnormal development of AM, which develops into ventral part of primary neural tube and notochord, could affect PNP closure. It is expected that development of the AM and its role in the development of the myeloschisis should be investigated by using animal models. It has been reported that the neural plate/tube is attached to the ventrally located medullay cord in chick embryos in their junctional region, similar

to AM in human embryos (Schoenwolf, 1979; Schoenwolf and Delongo, 1980; Saitsu et al., 2004). In addition, in this study, we demonstrated that the AM could differentiate the secondary neural tube containing cavities in embryos with myeloschisis, similarly to chick medullary cord. Thus, chick embryos appear to be suitable for studying the mechanism of the AM development and for examining whether abnormal differentiation of the AM is a cause of lumbosacral myeloschisis.

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