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REVIEW ARTICLE

Involvement of the axially condensed tail bud mesenchyme in normal and abnormal human posterior neural tube development

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ABSTRACT **Development of the posterior neural tube (PNT) in human embryos is a complicated process which involves both primary and secondary neurulation. Normal development of the human PNT should be understood to elucidate the pathogenesis of spinal neural tube defects, but there have been some discrepancies among previous reports. We examined histologically 20 human embryos around the stage of the posterior neuropore closure and found that the developing PNT can be divided into three parts: (1) the most rostral region which corresponds to the posterior part of the primary neural tube; (2) the junctional region of the primary and secondary neural tubes; and (3) the caudal region which emerges from the neural cord. In the junctional region, the axially condensed mesenchyme (AM) intervened between the neural plate/tube and the notochord. The AM appeared to be incorporated into the most ventral part of the primary neural tube, and no cavity was observed in the AM. Interestingly, we found three cases of human embryos with lumbosacral myeloschisis in which the open primary neural tube and the closed secondary neural tube overlapped dorso-ventrally. 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 embryos with lumbosacral 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 dorso-ventral overlapping of the primary and secondary neural tubes. These findings suggest that the AM in human embryos plays some role in normal and abnormal development of the human posterior neural tube.**

Key Words: axially condensed mesenchyme, human embryo, myeloschisis, primary neural tube, secondary neural tube

INTRODUCTION

In human embryos, the neural tube forms by means of two distinct developmental events: primary and secondary neurulation. During primary neurulation, the lateral ends of the neural plate elevate and the bilateral neural folds fuse with each other to form the primary neural tube (O'Rahilly & 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 & Müller 1994; Colas & Schoenwolf 2001; Müller & O'Rahilly 2004). Closure of the posterior neuropore occurs at the upper sacral level during Carnegie stage (CS) 12 (Müller & O'Rahilly 1987; Nakatsu *et al*. 2000; O'Rahilly & Müller 2003). Because of slow growth of the neural tube relative to that of vertebrae, the junction of the primary and secondary neural tubes is apposed at the lumbosacral level of the vertebral column in neonates (O'Rahilly & 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 0.5–2/1000 live births (Botto *et al*. 2006). Non-closure 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 & Müller 2003). Moreover, it has been suggested that skin-covered NTD, such as diplomyelia are caused by aberrant cavitation below the lower lumbar level of the spinal cord, which corresponds to the PNT (Dryden 1980; Lemire 1988). Thus, normal development of the PNT should be examined to understand pathogenesis of spinal NTD.

PNT DEVELOPMENT IN ANIMAL MODELS AND HUMAN EMBRYOS

Development of the PNT has been extensively studied in chick embryos. Multiple cavities are formed at the beginning of cavitation in the medullary cord, which differentiates from the tail bud (Criley 1969; Schoenwolf 1979; Schoenwolf & Delongo 1980). Enlargement and subsequent coalescence of the cavities result in a single cavity, which eventually becomes continuous with the central cavity of the primary neural tube. Notably, there is an overlapping zone where primary neurulation occurs dorsally and secondary neurulation ventrally. On the other hand, development of the PNT in mice appears to be simpler than in chicks (Hughes & Freeman 1974; Schoenwolf 1984; Nievelstein *et al*. 1993). Multiple cavities are not formed during the course of its development. Instead, the cavity of

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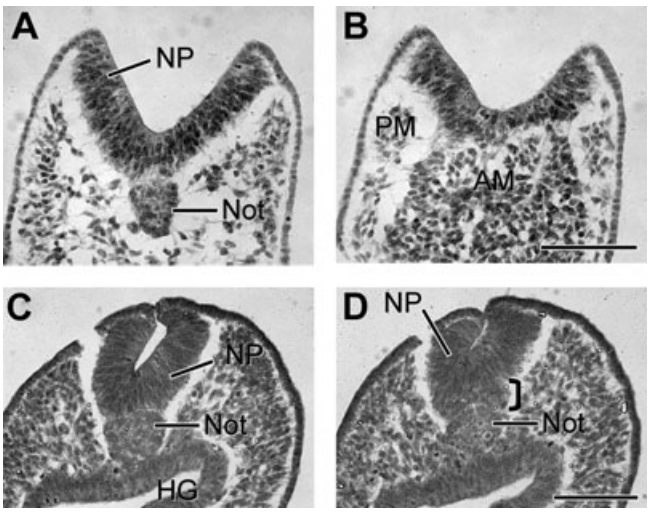

Fig. 1 (A,B) Transverse sections through the posterior part of an early Carnegie stage (CS) 12 embryo (No. 741). (A) At the level of the posterior neuropore, the notochord (Not) is attached to the neural plate (NP), which is widely open. (B) In a section approximately 80 μm more posterior to A, the notochord is not formed and the axially condensed mesenchyme (AM) is located beneath the neural plate. PM, paraxial mesenchyme. Bar, 100 μm. (C,D) Transverse sections of a CS 12 embryo (No. 589) at approximately 20 μm interval through the closing posterior neuropore. As the notochord is formed from the AM, the AM intervenes between the notochord and the neural plate (D, bracket), although the notochord is closely attached to the neural plate in a more rostral section (C). HG, hindgut. Bar, 100 μm. Saitsu *et al.* 2004.

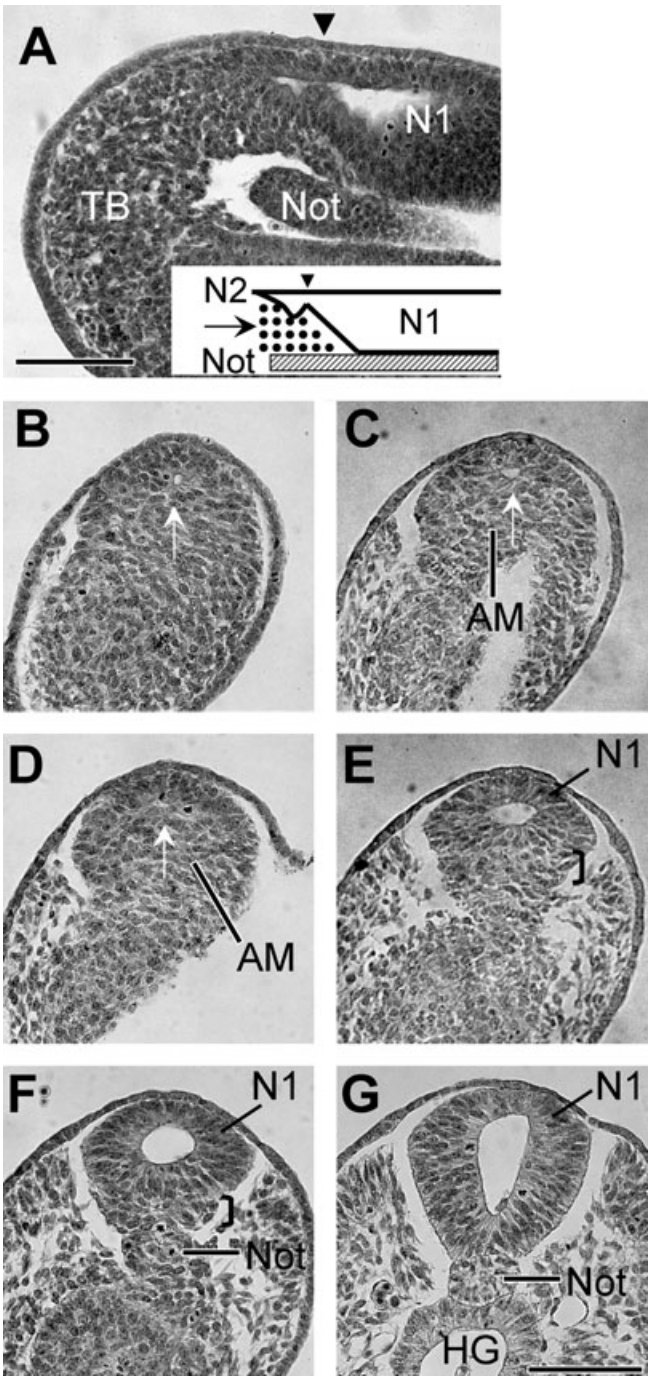

Fig. 2 Sections of the junctional region of the primary and secondary neural tubes in late Carnegie stage (CS) 12 embryos. (A) A sagittal section of No. 13087 embryo. Diagrammatic representation is shown in inlet of A. The notochord (Not) is attached to the primary neural tube (N1) along almost its entire length. As the primary neural tube tapers caudally, the axially condensed mesenchyme (AM) intervenes between the notochord and the primary neural tube (arrow in inlet). The cavity of the secondary neural tube (N2) is single and continuous with that of the primary neural tube. The junction between the primary and secondary neural tubes is indicated by a black arrowhead. TB, tail bud. Bar, 100 μm. (B–G) Serial transverse sections of No. 3005 embryo in a caudo-rostral sequence. (B–D) A small cavity of the secondary neural tube is surrounded by the radially arranged cells in AM (white arrows). (E,F) In more rostral sections, the size of the cavity is enlarged and AM intervenes between the notochord and the primary neural tube (brackets). (G) A section approximately 110 μm rostral to F. The notochord is attached to the primary neural tube in the most rostral section. HG, hindgut; Bar = 100 μm. Saitsu *et al.* 2004.

the primary neural tube extends continuously into the 'medullary rosette', which consists of elongated tail bud cells around the cavity. There is no overlapping zone of the primary and secondary neural tubes. Such species difference in the PNT development may account for the higher incidence of lumbosacral myeloschisis in chicks than in mice under various experimental conditions (Hughes & Freeman 1974; Schoenwolf 1979).

There have been discrepancies among reports concerning human PNT development. Müller and O'Rahilly (1987, 1988, 2004) reported that the cavity of the fully formed primary neural tube extended continuously into the tail bud and no isolated cavities were observed during secondary neurulation in human embryos. Other investigators claimed that multiple cavities were present in the human PNT as observed in the chick PNT (Bolli 1966; Lemire 1969; Hughes & Freeman 1974; Yasuda *et al*. 1985; Saraga-Babic *et al*. 1995). Therefore, normal development of the PNT in human embryos needs to be examined further in detail.

The Kyoto Collection of Human Embryos is the world's largest collection of human conceptuses and contains approximately 44 000 embryos derived mainly from healthy pregnancies. The details of the embryo collection and its demographic characteristics have been previously described elsewhere (Nishimura 1975;

Matsunaga & Shiota 1977; Shiota 1991). The embryo collection includes a large number of human embryos at the stage of neurulation, as well as an appreciable number of embryos associated with neural malformations including myeloschisis. Some of the well-preserved embryos were photographed and serially sectioned at a thickness of 10 μm for histological examination. By using these histological preparations, we examined 20 cases of externally normal human embryos at CS 12 and 13, and three embryos with lumbosacral myeloschisis at CS 16, 18 and 20 with special reference to development of the PNT (Saitsu *et al*. 2004, 2007).

THE AM INTERVENES BETWEEN THE NEURAL PLATE/TUBE AND THE NOTOCHORD IN THE JUNCTIONAL REGION OF THE PRIMARY AND SECONDARY NEURAL TUBES

In a CS 12 embryo with its posterior neuropore widely open, the notochord was attached to the neural plate (Fig. 1A). At the posterior end of the neural plate, the notochord was not formed and the axially condensed mesenchyme (AM) derived from the tail bud (Müller & O'Rahilly 1986, 2004) was located beneath the neural plate (Fig. 1B). In more advanced embryos with the posterior neuropore closing (CS 12), as the notochord was formed from the AM, the AM intervened between the notochord and the neural plate (Fig. 1D), although the notochord was closely attached to the neural plate at the rostral part of the posterior neuropore (Fig. 1C). Slightly advanced embryos, in which the posterior neuropore had closed, also showed that the AM was still present between the notochord and the primary neural tube (Fig. 2A,E,F). In a sagittal section of a CS 12 embryo, the notochord was in close contact with the neuroepithelium at the most part of the primary neural tube. As the primary neural tube became tapered caudally, AM appeared to intervene between the notochord and the primary neural tube in the junctional region (Fig. 2A). This feature was also observed in transverse sections of the same stage embryo (Fig. 2E,F). A small cavity in the AM seemed to be a cavity of the secondary neural tube (Fig. 2B–D, white arrows). The size of the cavity became enlarged rostrally and the AM intervened between the notochord and the primary neural tube (Fig. 2E,F) while the primary neural tube was closely attached to the notochord in the most rostral region of the PNT (Fig. 2G). Since the AM appeared to be incorporated into the most ventral part of the neural tube (Fig. 2E,F), it is likely that both the primary neural tube and AM participate in the formation of the PNT in the junctional region. The feature of the AM intervened between the neural plate/tube and the notochord in the junctional region, as was first described in our report (Saitsu *et al*. 2004), is discussed later.

THE CAVITY OF THE EARLY SECONDARY NEURAL TUBE IS CONTINUOUS WITH THAT OF THE PNT IN THE JUNCTIONAL REGION

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 (Fig. 2A–D). No cavity was observed under the cavity of the primary neural tube contrary to in the chick medullary cord (Figs 1D,2A–F). These observations are consistent with the previous report by Müller and O'Rahilly (1987) in which 24 embryos at CS 12 were examined. They demonstrated that the cavity of the secondary neural tube was continuous with that of the primary neural tube and was surrounded by radially arranged cells as observed in mice. Therefore, in terms of cavity formation, the transition from the primary to the secondary neural tubes and the early developmental features of the secondary neural tube in the junctional region are similar to those in mice.

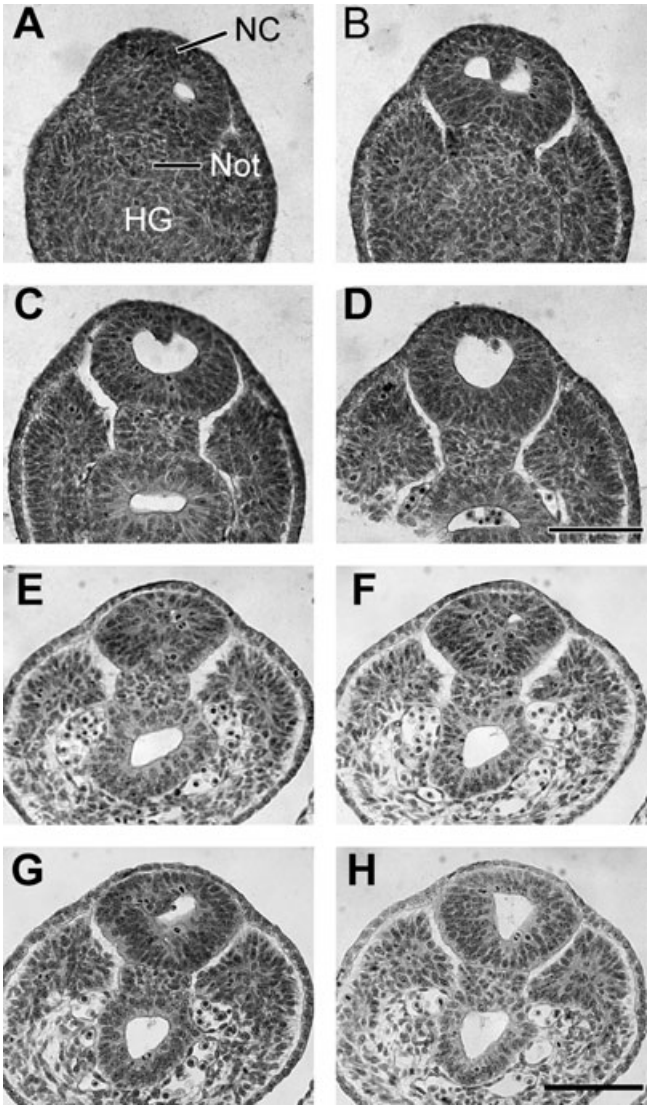

Fig. 3 Sections of the caudal region of Carnegie stage (CS) 13 embryos. Multiple cavities are formed in the neural cord (NC), which is attached to the notochord (Not). (A–D) Serial transverse sections of No. 584 embryo at approximately 20 μm intervals in a caudo-rostral sequence. The septum between the laterally located two cavities (B) disappears in more rostral sections, forming in a single central cavity (C,D). A small mass of cells protrudes from the dorsal wall of the neural tube into the central cavity in C. (E–H) Serial transverse sections of the caudal region of a CS 13 embryo (No. 14205) at approximately 30 μm (E, F) and 20 μm intervals (G,H) in a caudo-rostral sequence. As the centrally located cells disappear, the cavities seem to enlarge and/or coalesce with each other to form a single central cavity which becomes continuous with the cavity of the primary neural tube. HG, hindgut; Bar, 100 μm. Saitsu *et al.* 2004.

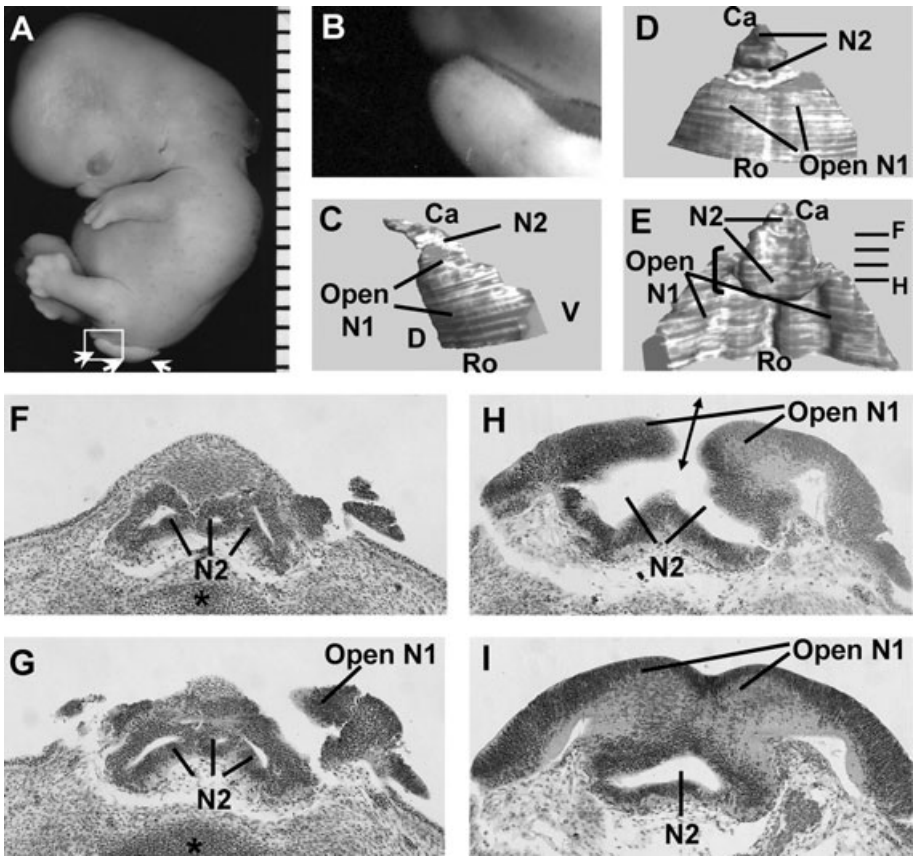

Fig. 4 (A) CS 20 embryo with lumbosacral myeloschisis (No. 18289). (A) Lateral view of the embryo. The open neural tube is observed caudal to the level of the hind limb (white arrows). (B) Close-up lateral view of the myeloschisis region is indicated by white box in figure part A. (C) The neural tubes in this region are 3D-constructed (compare C with B). In the lateral (C) and dorsal (D) view, the secondary neural tube (N2) extends caudally from the open primary neural tube (open N1). In addition, in the ventral view (E), a dorso-ventral overlapping between the open primary and closed secondary neural tubes is clearly observed (bracket). Ca, caudal; D, dorsal; Ro, rostral; V, ventral. (F–I) 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 H), and become separated from the primary neural tube to form a distinct lumen ventral to the open primary neural tube (I). The secondary neural tube shows multiple cavities (F,G) as is shown in Figure 3. The vertebral body is indicated by asterisk (F,G). Bar, 200 μm (F–I). Saitsu *et al.* 2004.

CAVITIES OF THE SECONDARY NEURAL TUBE IN THE CAUDAL REGION ARE FREQUENTLY FORMED AT MULTIPLE SITES IN THE NEURAL CORD

The caudal region of the secondary neural tube predominantly differentiated from the neural cord which is derived from the tail bud (Müller & O'Rahilly 1988) (Fig. 3). In the neural cord, it appeared that cavitation was not a simple extension of the cavity of the primary neural tube as was the case in the junctional region (compare Fig. 3 with Fig. 2). Contrary to the previous report by Müller and O'Rahilly (1988), multiple cavities were frequently observed in CS 13 embryos (Fig. 3) (Saitsu *et al*. 2004). Cavitation seemed to occur off the center, not at the position of the presumptive central cavity. As the centrally located cells disappeared, the cavities seemed to enlarge and/or coalesce with each other to form a single central cavity which becomes continuous with the cavity of the primary neural tube (Fig. 3). Because cavitation occurs between peripheral and central cells in the chick medullary cord (Schoenwolf & Delongo 1980), these findings suggest that cavitation in the neural cord which occurs at a later stage of secondary neurulation resembles, at least in part, that in the chick medullary cord. Previous investigators who claimed that multiple cavities were present in the human PNT examined between CS 14 and 21 (Bolli 1966; Lemire 1969; Hughes & Freeman 1974; Saraga-Babic *et al*. 1995). Thus, all these previous studies together with ours suggest that multiple cavities are first formed in the neural cord of human embryos (Saitsu *et al*. 2004).

ABERRANT DIFFERENTIATION OF THE AM CELLS IN HUMAN EMBRYOS WITH LUMBOSACRAL MYELOSCHISIS

One noticeable observation is that the AM intervened between the notochord and the neural plate/tube in the junctional region of the primary and secondary neural tubes in human embryos. This feature is similar to that in chick embryos in which the neural plate/tube is attached to the ventrally located medullary cord in their overlapping zone. It has been suggested that the high incidence of lumbosacral myeloschisis in chicks may be related to the existence of the overlapping zone of the primary and secondary neural tubes described above (Schoenwolf 1979). Although no cavity was observed in the AM beneath the primary neural tube in human embryos, a similar relationship between the neural plate/tube and the AM (medullary cord in chicks) in the junctional region suggests that AM may play some role in the development of human myeloschisis. In the Kyoto Collection of Human Embryos, we encountered three embryos with lumbosacral myeloschisis in which the open primary neural tube and the closed secondary neural tube overlapped dorso-ventrally (Fig. 4) (Saitsu *et al*. 2007). In a CS 20 embryo among the three embryos, the open neural tube was observed caudal to the level of the hind limb and the exposed neural tissue appeared overgrown (Fig. 4A, white arrows). In a ventral view of the 3D image of the neural tubes reconstructed by delta viewer software (Yamada *et al*. 2007), the closed secondary and open primary neural tubes partially overlapped at their junction (Fig. 4E, bracket). As illustrated on caudal-rostral series of sections (Fig. 4F–I), the lumens of the

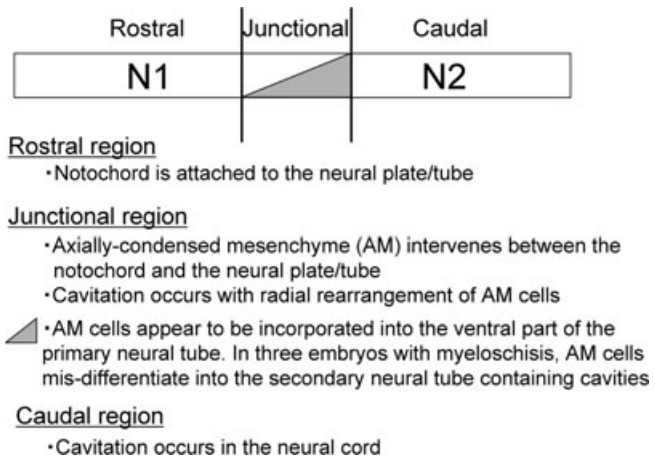

Fig. 5 Summary scheme of posterior neural tube (PNT) development in normal human embryos and the difference in the axially condensed mesenchyme (AM) between normal embryos and three embryos with lumbosacral myeloschisis. The posterior neural tube can be divided into three parts. In the most rostral region, the notochord is directly attached to the neural plate/tube, while, in the junctional region, the AM intervenes between the notochord and the neural plate/tube. In normal embryos, the AM appears to be incorporated into the ventral part of the primary neural tube. On the other hand, the AM appears to differentiate aberrantly to form the secondary neural tube containing cavities in the three embryos with myeloschisis. Cavitation in the junctional region occurs with radial rearrangement of AM cells. A single cavity is formed in the junctional region. In contrast, the cavitation of the secondary neural tube occurs in the neural cord in the caudal region. Multiple or isolated cavities are frequently observed in the neural cord. N1, primary neural tube; N2, secondary neural tube.

secondary neural tube only partly connected to the open lumen of the primary neural tube (Fig. 4H, 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. 4I). The secondary neural tube had multiple cavities (Fig. 4F,G), which is consistent with the idea that multiple cavities are formed in the neural cord of human embryos (Fig. 3) (Saitsu *et al*. 2004). It was noteworthy that the closed secondary neural tube was intercalated between the open neural tube and the developing vertebral body, which surrounded the notochord (Fig. 4F,G), 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). 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 dorso-ventral overlapping of primary and secondary neural tubes. Such abnormal differentiation of the AM in embryos with lumbosacral myeloschisis suggest that the AM plays some role in abnormal development of the human posterior neural tube in the junctional region.

ROLE OF VENTRAL TISSUES SUCH AS AM AND HINDGUT IN HUMAN MYELOSCHISIS

Posterior neural tube development in normal human embryos and the difference in the AM between normal embryos and three embryos with lumbosacral myeloschisis are summarized in Figure 5 (Saitsu *et al*. 2004, 2007). AM intervenes between the notochord and the neural plate in the junctional region of the primary and secondary neural tubes corresponding to the lumbosacral region in human neonates (O'Rahilly & Müller 2003). Considering the common occurrence of the myeloschisis in the lumbosacral region, the AM seems to be involved in pathogenesis of myeloschisis. In fact, some human embryos with lumbosacral myeoloschisis showed abnormal development of the AM in the junctional region. It is noteworthy that spinal NTD in *curly tail* mice result from a defective proliferation of hindgut cells resulting in excessive ventral curvature of the caudal region of the embryo and preventing the neural folds from closing (van Straaten & Copp 2001). This fact suggests that a dorso-ventral cell proliferation imbalance could be a cause of human myeloschisis. Therefore, it would be possible that abnormal development of AM, which develop into ventral part of primary neural tube and notochord, could affect the posterior neurpore closure, leading to myeloschisis. Interestingly, it has been recently reported that polymorphisms in *ALDH1A2*, aldehyde dehydrogenase required for synthesis of retinoic acid, may influence the risk for lumbosacral myelomeningocele in humans (Deak *et al*. 2005). In addition, one frameshift mutation has been found in *CYP26A1*, cytochrome P450 enzymes involved in metabolism of retinoic acid, in a patient with spina bifida (Rat *et al*. 2006). Because spinal NTD in *curly tail* mice could be prevented by low dose retinoic acid supplementation (van Straaten & Copp 2001), these facts together with our histological findings suggest that ventrally located tissues such as AM and hindgut may play some important roles in pathogenesis of human myeloschisis.

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