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RESEARCH ARTICLE

Developmental processes and ectodermal contribution to the anal canal in mice

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Summary

The anorectal canal has two origins; the upper part is derived from endoderm and the lower part is derived from ectoderm. The process of ectodermal contribution to the canal remains unclear. To understand the development of this area, serial sagittal sections of mouse embryos were made every 12 h from embryonic day 13.0 (E13.0) to E18.5. Three-dimensional (3-D) reconstructions were obtained from these sections. At the time of the disappearance of the cloacal membrane (E13.5), the endodermal lining reached the site of disintegrated membrane. Thus, the whole canal was of endodermal origin. The transitional zone between the dorsal end of the primary perineum and tail was thicker than other ectodermal epithelia. In this region, it changed from an acute to obtuse angle. After it straightened out and formed the canal, the secondary perineum appeared caudally. During these processes, the external sphincter appeared in the underlying mesenchyme of the thick ectoderm and functioned as a drawstring to form the ectodermal anal canal.

Introduction

Despite a long history of embryological research, the developmental processes of the anorectal canal remain matters of controversy. The most debated point has concerned the separation of the urogenital sinus and the anal canal, in particular, the question of whether or not fusion of the urorectal septum with the cloacal membrane occurs in normal development (Keibel, 1895; Pohlman, 1911; Politzer, 1931; DeVries and Friedland, 1974a, b; Van der Putte and Neeteson, 1983; Van der Putte, 1986; Stephens and Smith, 1988; Kluth et al., 1995; Nievelstein et al., 1998; Kromer, 1999; Paidas et al., 1999; Qi et al., 2000a–c; Sasaki et al., 2004).

In our previous research on the cloacal development and formation of the urogenital sinus and anorectal canal, fusion of the urorectal septum with the cloacal membrane was not observed (Sasaki et al., 2004). Thus, the ventral and dorsal

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parts of the cloaca were continuously connected via the canal until disappearance of the cloacal membrane to open the vestibule formed by the urogenital sinus and anorectal canal to the outside at E13.5.

The anal canal is known to be derived embryonically from two sources. The region above the anal valves arises from the endodermally lined cloaca, while, below the valve, it comes from the proctodeum covered with ectoderm. The junction of ectodermal and endodermal parts has been suggested to be at the lower border of the pecten (Johnson, 1914). However, the ectodermal contribution to the canal remains unclear.

In classical comparative studies of the cloacal sphincter with special reference to nerve supply, some researchers have postulated that this sphincter probably originated from a limb muscle (Nishi, 1938; Akita, 1992a, b; Akita et al., 1992, 1995). They postulated that proximal ventral muscles of the hind limb migrated towards the tail to make the anal sphincter muscles. Recently, Valasek et al. (2005) confirmed that perineal muscles of chick and mice are derived from the ventral muscle mass of the hind limb.

In the present study, we used mouse embryos to observe the developmental processes of the rectum and the anal canal. We also analyzed the process of formation of perineal muscles, especially the external sphincter muscle during the maturation of the anal canal.

Materials and methods

Animal and tissue preparation for light microscopy

Mature female ICR mice (SLC, Shizuoka, Japan) were mated overnight with a male mouse. The morning of the day on which a vaginal plug was found was designated as E0.5. For sequential examination of normal development in the pelvic region, two pregnant mice were sacrificed every 12 h from E13.0 to E18.5 ($n = 24$). Handling of animals conformed to the Guidelines for Care and Use of Experimental Animals as established by the Institutional Animal Care and Use Committee of Tokyo Medical and Dental University (No. 0070043). Embryos were fixed in 10% formalin, dehydrated, and embedded in paraffin. A total of 48 embryos were serially sectioned in the sagittal plane (5 μm thickness). For two embryos at each stage, the sections were stained with hematoxylin and eosin (HE). For the other two embryos at each stage, the sections were alternately stained with HE and other stains.

In order to confirm the distribution of skeletal muscle, immunohistological staining was performed with an anti-skeletal myosin antibody [monoclonal anti-skeletal myosin (fast) clone MY-32 mouse ascites fluid (product No. M4276, Sigma, St. Louis, MO, USA)]. Combined antibodies were stained by diaminobenzidine with Histostain-SP kit (Zymed, San Francisco, CA, USA).

Three-dimensional reconstructions

Endodermal and ectodermal epithelium, smooth muscle layer of the intestine and striated muscle were analyzed using computer-assisted three-dimensional (3-D) reconstruction. 3-D reconstructions were made using one embryo at each stage. All of the serial sections were photographed and the endodermal epithelium was traced in yellow, ectodermal epithelium in blue, smooth muscle layer in pink, and external anal sphincter in red. Section sequences were reconstructed using SrfII software (SrfII, Ratoc, Tokyo, Japan).

Results

Analysis of the median sagittal sections

In mice, the cloaca was completely divided into the urogenital sinus and anorectal canal by E13.5. At this stage of development, the intestinal wall was in contact with the perineum, and there was no space for anal sphincter muscles. To understand the developmental process of the anorectal canal, we examined normal development of the pelvic region sequentially using serial sagittal sections of mouse embryos from E13.0 to E17.5. First, we describe the morphology of the anal canal and the surrounding structures of mouse embryo in the median sagittal plane (Figures 1, 2).

At E13.0, the cloaca was covered by only a thin cloacal membrane (Figures 1A, 2A). This membrane consisted of a single cell layer epithelium, containing apoptotic cells. The urogenital sinus and hindgut were nearly completely separated by the urorectal septum. However, the urogenital sinus and distal end of the hindgut, i.e., the ventral and dorsal parts of the cloaca in the earlier stages, were completely connected to each other. The epithelium of the hindgut was composed of simple columnar cells with oval-shaped nuclei, and eosinophilic cytosol (intestinal epithelium, Figure 2A). At the tip of the septum, these intestinal epithelial