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RESEARCH ARTICLE

Smooth-to-striated muscle transition in human esophagus: An immunohistochemical study using fetal and adult materials ☆

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ABSTRACT

Background: A craniocaudal transition from smooth to striated muscle occurs in the fetal mouse esophagus muscularis propria, until finally the entire muscle component becomes striated. Although no such investigation has been conducted using human fetuses, the transition appears to be incomplete.
Methods: In horizontal sections of 10 human fetuses between 9 and 16 weeks of gestation, we identified immunoreactivity for smooth muscle actin (SMA), striated muscle myosin heavy chain (MyH), desmin, PGP9.5, S100 protein, c-kit, and CD68 in the thoracic esophagus. The TUNEL method was used to identify apoptosis. For comparison, the same immunohistochemistry was conducted using 10 adult esophaguses.
Results: In fetuses at all stages examined, a transition zone was found in the upper thoracic esophagus that was attached to the middle one-third of the trachea. In the transition zone, the MyH-positive longitudinal muscle fibers were surrounded by flat, SMA-positive cells, whereas the MyH-positive circular fibers were sometimes located adjacent to the SMA-positive fibers. However, in adults, smooth muscle tended to be clearly separated from striated muscle. The distribution of cells showing immunoreactivity for PGP9.5, S100 or c-kit did not differ between the oral and anal sides of the transition zone. Desmin was positive in the muscularis propria, but negative in the muscularis mucosae. Neither CD68-positive macrophages nor TUNEL-positive cells were present in the esophagus.
Conclusions: In the human esophagus, the smooth-to-striated muscle transition appears to stop at the mid-thoracic level. Cell death or transdifferentiation of smooth muscle appears unlikely, but phenotypic transformation into desmin-positive myofibroblasts is a possibility.

1. Introduction

In the mouse esophagus, the tunica muscularis propria or externa (the external longitudinal and internal circular muscle layers) initially develops as smooth muscle during the early embryologic stage, but at day 14 of gestation striated muscle appears in the cranial part and external layer of the esophagus, and subsequently replaces the smooth muscle in a caudal direction as well as toward the internal layers. Patapoutian et al. (1995) were the first to report this process, and considered it a rare example of transdifferentiation with a phenotypic switch. Thereafter, it has been described and examined by many research groups (Kabler et al., 2000; Reddy and Kabler, 2004; Stratton et al., 2000; Wörl and Neuhuber, 2000; Zhao and Dhoot, 2000a; Zhao and Dhoot, 2000b). The mouse esophageal muscularis propria remains completely striated until 14 days after birth, whereas the human esophagus is composed of a mixture of smooth and striated muscle in the middle one-third and of proper smooth muscle in the lower one-third (Standring, 2005; Goyal and Chaudhury, 2008), the latter arrangement being established at 5 months of gestation (DeNardi and Riddell, 1997). However, to our

Abbreviations: MyH, striated muscle myosin heavy chain; SMA, smooth muscle actin; TUNEL method, terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end-labeling method

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knowledge, no study has investigated the transition of smooth to striated muscle in the human fetal esophagus.

Early studies of the mouse esophagus discussed whether or not the smooth-to-striated muscle transition occurs through transdifferentiation. However, recent studies (Rishniw et al., 2003; Rishniw et al., 2007; Rishniw et al., 2008) have led to an established concept that no transdifferentiation occurs; there are distinct differentiation pathways for smooth and striated muscle. Thus, during the transition process, apoptosis of smooth muscle cells is considered to occur in parallel to striated myogenesis, although for some reason identification is difficult using the terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end-labeling (TUNEL) method (reviewed by Wörl and Neuhuber, 2005). In addition, it is still uncertain whether or not nerve elements, including the interstitial cells of Cajal, are committed to a change of muscle phenotype during muscle transition in the mouse esophagus (Reddy and Kabler, 2004; Rishniw et al., 2008; Breuer et al., 2004; Sang and Young, 1997).

Consequently, the aim of the present study was to investigate the esophageal histology of the transition zone between smooth and striated muscle in the human fetus using immunohistochemistry for smooth muscle actin (SMA), striated muscle myosin heavy chain (MyH), desmin (a marker of intermediate cytoskeletal filaments), PGP9.5 (a pan-neuronal marker), S100 protein (a marker of Schwann cells), CD68 (a marker of a major macrophage population), and c-kit (a marker of the interstitial cells of Cajal). The macrophage marker was used for indirect identification of dead cells. However, the TUNEL method was also used to detect any apoptosis of smooth muscle cells. In addition, we compared the fetal histology with specimens obtained from elderly human cadavers.

2. Materials and methods

In accordance with the provisions of the Declaration of Helsinki, 1995 (as revised in Edinburgh 2000), we examined 10 fetuses at stages between 9 and 16 weeks of gestation (6 males and 4 females; CRL, 35–110 mm): 2 fetuses at 9–10 weeks; 2 at 12–13 weeks; 6 at 15–16 weeks. With the agreement of the families concerned, these fetuses had been donated to the Department of Anatomy, Chonbuk National University, Korea, and use of the fetuses for research had been approved by the university ethics committee. All specimens appeared normal on the basis of gross inspection, and had been fixed in 10% formalin solution, decalcified using EDTA (Decalcification Solution B, Wako, Tokyo), and paraffin-embedded for histological examination. Horizontal paraffin sections 5 μm thick were cut serially. Most of these sections were used for hematoxylin and eosin staining, but some were used for immunohistochemistry (see below). Although the cervical, lower thoracic and abdominal parts of the fetal esophagus were also examined, the present investigation was focused on the mid- and upper-thoracic levels, as these included the smooth-to-striated muscle transition zone.

The adult specimens were obtained from 10 elderly cadavers (5 males and 5 females; mean age 75 years) that had been donated to Sapporo Medical University for education and research: these specimens (paraffin blocks) were overlapped with those used in another histological study of the esophageal lymphatic vessels (Yajin et al., 2009). In all cases, the cause of death was brain infarction or acute myocardial infarction. From one esophagus, several horizontal sections were dissected at 2 sites: the upper thoracic esophagus above the aortic arch and the middle thoracic esophagus immediately below the tracheal bifurcation. The use of donated adult cadavers for anatomical research did not require examination and approval by a suitably constituted institutional ethics committee.

The primary antibodies used were (1) monoclonal anti-human alpha-1 smooth muscle actin or SMA (dilution 1:100, Dako, Glostrup, Denmark); (2) monoclonal anti-human striated muscle myosin heavy chain or MyH (dilution 1:100, Dako); (3) monoclonal anti-human desmin (dilution 1:100, Dako); (4) monoclonal anti-human S100 protein (dilution 1:100, Dako Cytomation, Kyoto, Japan), (5) polyclonal anti-human PGP9.5 (ubiquitin carboxyl-1-terminal hydrolase or protein gene product 9.5; dilution 1:400, KosmoBio, Tokyo, Japan); (6) monoclonal anti-human c-kit (dilution 1:100, Dako); (7) monoclonal anti-human CD68 (dilution 1:100, Dako). All these antibodies were generated using the rabbit. Pretreatment for paraffin sections, such as microwave, was not performed. According to Gerecht-Nir et al. (2004) and Hayashi et al. (2008), Dako SMA antibody reacts with the endothelium of arteries and veins as well as any smooth muscle cells, but is non-reactive to lymphatic endothelium. Using Dako EnvisionChemMate, the second antibody was labeled with horseradish peroxidase (HRP) and antigen–antibody reactions were detected using the HRP-catalyzed reaction with diaminobenzidine (with hematoxylin counterstaining). The intermediate filament desmin immunoreactivity is generally known to be expressed in skeletal muscle, myofibroblasts and pericytes. It often coexists with SMA in human tumor cells (Dundr et al., 2009; Farah-Klibi et al., 2008; Tai et al., 2008; Abraham et al., 2007; Alvarado-Cabrero et al., 2007) as well as in normal tissues (Xueyong et al., 2008; Tang, 2008; Kreplak and Fudge, 2007). Apoptotic cell nuclei were tested for DNA fragmentation by the TUNEL method (*in situ* apoptosis detection kit; Takara Biochemicals, Tokyo) using diaminobenzidine as the chromogen.

3. Results

3.1. Topographical anatomy and general observations of the fetal esophagus

At 9–12 weeks, the first thoracic vertebra (T1) was located at the level of the tracheal bifurcation or 50–100 μm inferior to it. Esophageal striated muscle was restricted to an area immediately inferior to the larynx. Therefore, we did not find any immunoreactivity for striated muscle myosin heavy chain (MyH) at the T1 level or in the "mid-thoracic" esophagus (Fig. 1C). At this level, the membranous part of the trachea as well as the muscularis propria of the esophagus was positive for both smooth muscle actin (SMA) and desmin (Figs. 1AB). However, desmin immunoreactivity was restricted in the internal circular layer. The thickness of the muscularis propria was largely made up by the internal circular layer because the external longitudinal layer was very thin. In the external layer, smooth muscle fibers appeared to be circular as in the internal layer (Figs. 1A and 2A). The muscularis mucosae was still not well developed at the thoracic level (Figs. 1A and 2A).

At 15–16 weeks, pharyngeal and laryngeal striated muscle was well differentiated: the posterior cricoarytenoideus communicated with the anterior part of the esophageal striated muscle, whereas the inferior pharyngeal constrictor communicated with the lateral part of the esophagus (figures, not shown). The inferior boundary of MyH-immunoreactivity extended inferiorly and reached the level of the first or second thoracic vertebra (Fig. 3B). This level corresponded to the apex of the lung, extending much more superiorly to the tracheal bifurcation at this stage. Thus, the tracheal bifurcation was still adjacent to the smooth muscle-dominant area of the esophagus. Desmin-positive cells were seen in the internal circular as well as the external longitudinal layer (Figs. 4CH). The muscularis mucosae, which was convenient for use