Annals of Anatomy 192 (2010) 33–41

Contents lists available at ScienceDirect

Annals of Anatomy

journal homepage: www.elsevier.de/aanat

RESEARCH ARTICLE

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

Yukio Katori [a,b,*], Baik Hwan Cho [c], Chang Ho Song [d], Mineko Fujimiya [e], Gen Murakami [f], Tetsuaki Kawase [a,g]

[a] Department of Otolaryngology & Head and Neck Surgery, Tohoku University Graduate School of Medicine, Sendai, Japan
[b] Division of Otorhinolaryngology, Sendai Municipal Hospital, Sendai, Japan
[c] Department of Surgery, Faculty of Medicine, Chonbuk National University, Jeonju, Korea
[d] Department of Anatomy, Faculty of Medicine, Chonbuk National University, Jeonju, Korea
[e] Department of Anatomy, Sapporo Medical University School of Medicine, Sapporo, Japan
[f] Division of Internal Medicine, Iwamizawa Koujin-kai Hospital, Iwamizawa, Japan
[g] Laboratory of Rehabilitative Auditory Science, Tohoku University Graduate School of Biomedical Engineering, Sendai, Japan

ARTICLE INFO

Article history:
Received 9 June 2009
Received in revised form
21 September 2009
Accepted 22 September 2009

Keywords:
Esophagus
Smooth muscle
Striated muscle
Transdifferentiation
Human fetus

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

☆ This study was supported by a grant-in-aid from the Japanese Ministry of Education, Culture, Sports, Science and Technology for fiscal 2008.

* Corresponding author at: Division of Otorhinolaryngology, Sendai Municipal Hospital, 3-1 Shimizu-kouji, Wakabayashi-ku, Sendai 984-8501, Japan.
Tel.: +81 22 266 7111; fax: +81 22 211 8972.
E-mail address: yukatori@concerto.plala.or.jp (Y. Katori).

0940-9602/$ - see front matter © 2009 Elsevier GmbH. All rights reserved.
doi:10.1016/j.aanat.2009.09.007

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

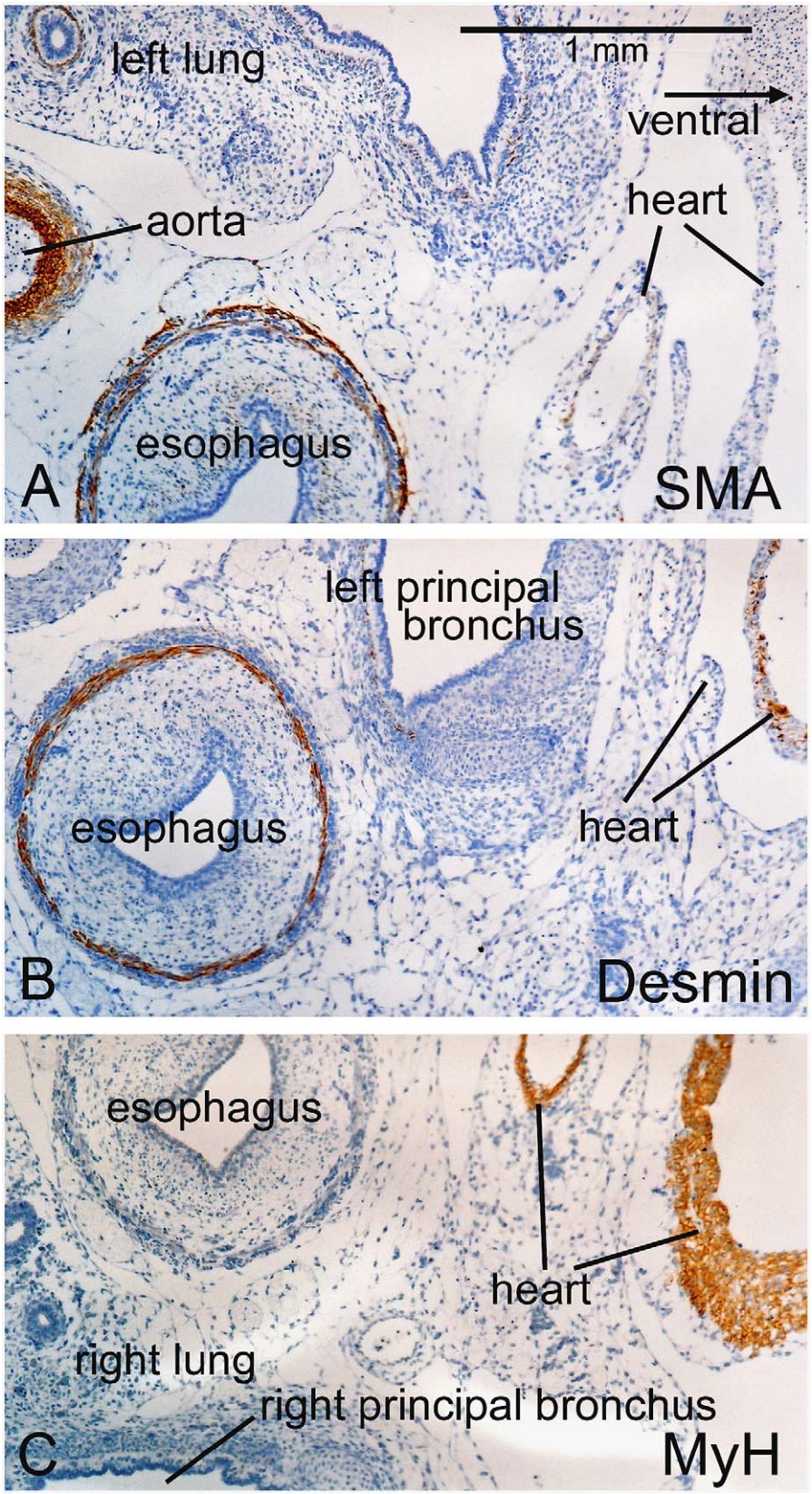

Fig. 1. Horizontal sections of mid-thoracic fetal esophagus at 11 week of gestation. Panel A (smooth muscle actin or SMA immunohistochemistry), panel B (desmin, one of intermediate filaments) and panel C (myosin heavy chain, MyH, striated muscle staining) display near sections at a level slightly below the tracheal bifurcation (panel B was the superiormost, while panel C inferiormost: distance between panels, 0.1 mm). SMA immunoreactivity was positive in the muscularis propria of the esophagus, vascular endothel (see Section 2) and muscular layer of the aorta, thick bronchi and parts of the heart. The muscularis mucosae was weekly positive for SMA. Desmin immunoreactivity was strongly positive for striated muscle, but weakly positive for smooth muscles, including the membranous part of the trachea and thick bronchi. Note, in panel B, the negative immunoreactivity of the external longitudinal layer and muscularis mucosae for desmin: (cf. Figs. 4C and H). MyH was positive for most parts of the heart. Those 3 panels were prepared at the same magnification (scale bar in panel A).

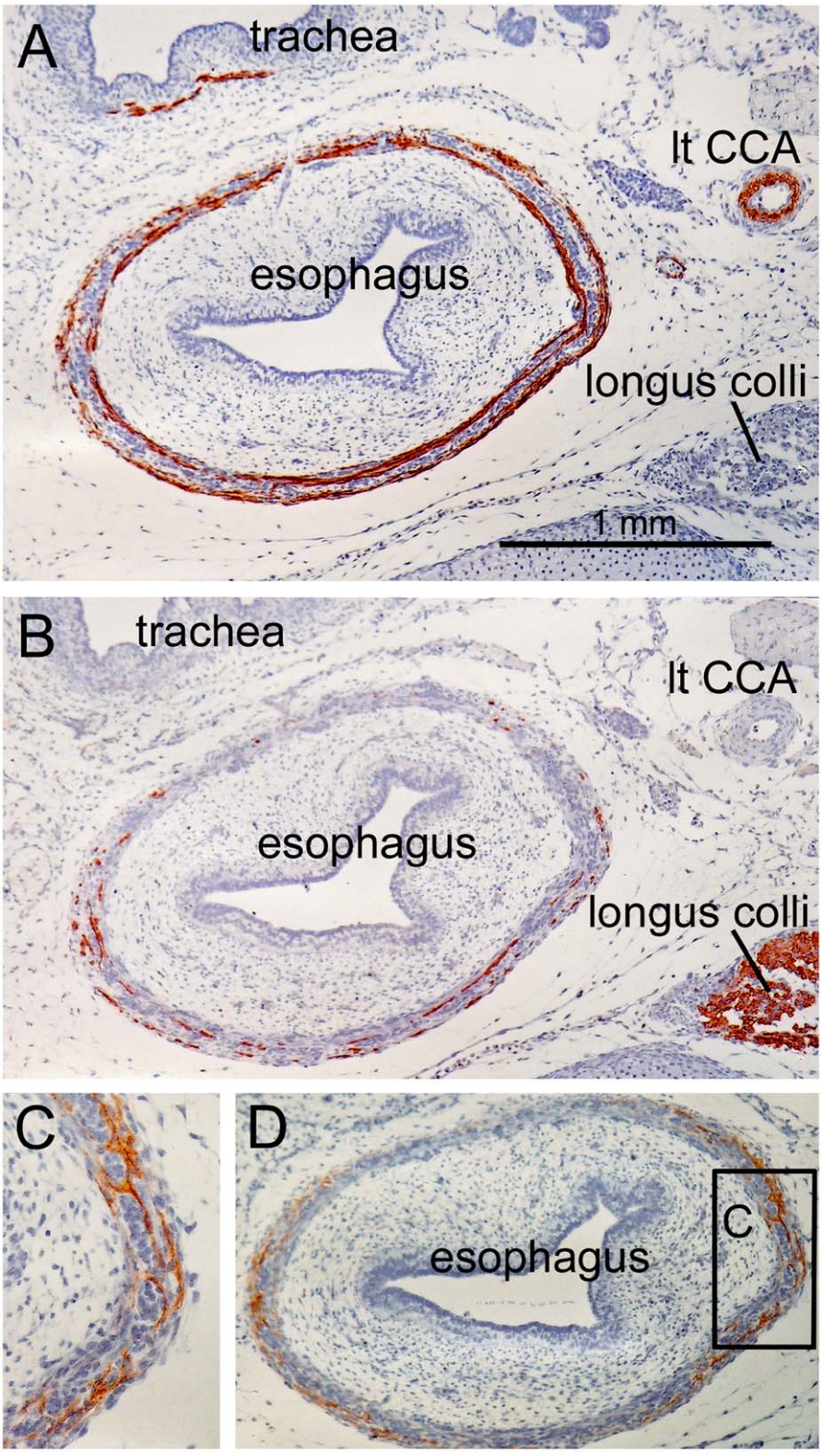

Fig. 2. Transitional zone between the smooth and striated muscles of the esophagus in a fetus at 12 weeks of gestation. Panel A (smooth muscle actin or SMA immunohistochemistry), panel B (myosin heavy chain, MyH, striated muscle staining), panels C and D (c-kit, interstitial cell marker) display near sections at a level immediately above the aortic arch (the upper thoracic esophagus). SMA immunoreactivity was seen in the membranous part of the trachea and left common carotid artery (lt CCA) as well as the esophagus. An endothelium of a vein is also positive (arrow). However, the muscularis mucosa is still negative. The longus colli muscle was strongly positive for MyoH immunoreactivity. Striated muscles are restricted in the posterior half of the esophagus. Most of the esophagus wall carries c-kit-positive cells except for the ventral part. Panel C is a higher magnification view of the left part (a square with C) of panel D. Panels A, B and D were prepared at the same magnification (scale bar in panel A).

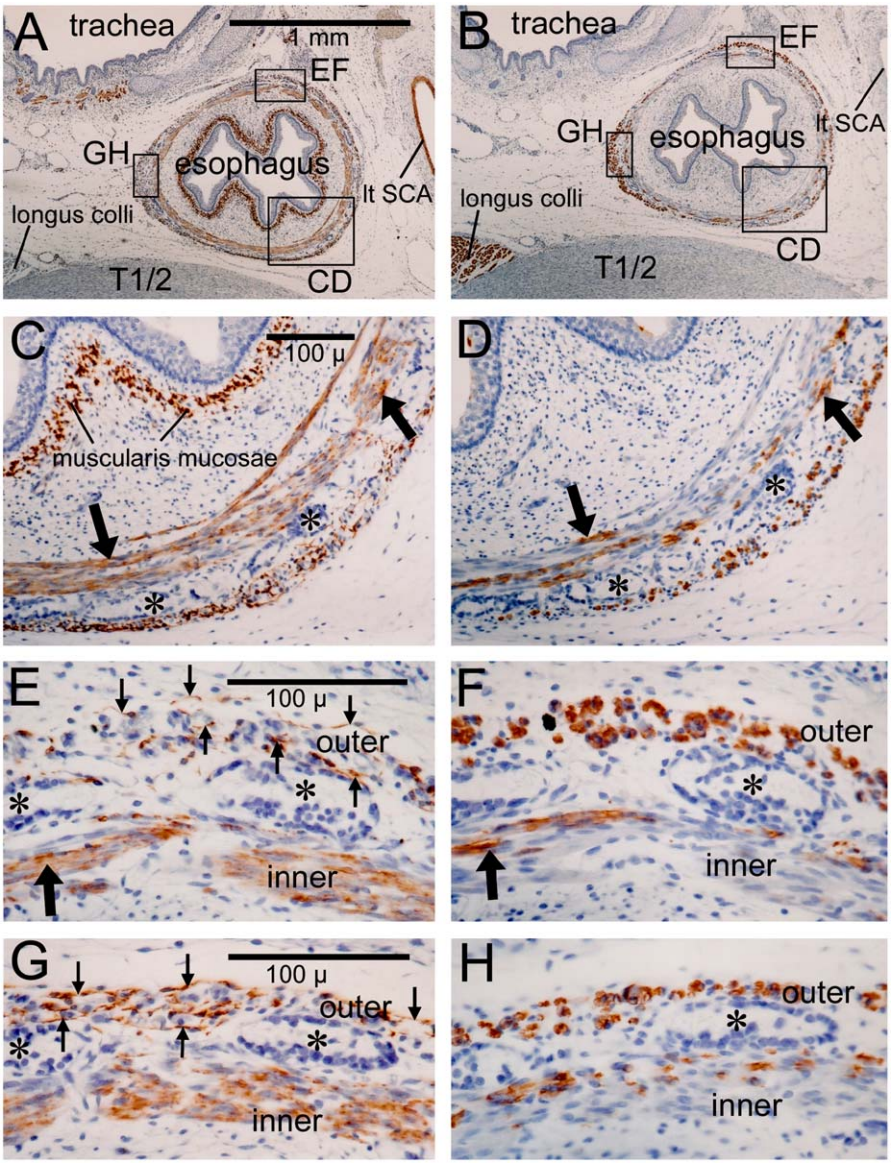

Fig. 3. Transitional zone between the smooth and striated muscles of the esophagus in a fetus at 15 weeks of gestation: observations using adjacent sections. The left-hand side half of the figures or panels A, C, E and G exhibits/exhibit SMA immunohistochemistry, while the right-hand side half or panels B, D, F and H displays/display MyH immunohistochemistry. A pair of the left- and right-hand sides (panels AB, CD, EF and GH) exhibits/exhibit the adjacent sections and was/were provided at the same magnification (scale bar, panels in the left-hand side). Panels A and B are the lower magnification views of the transitional zone at the level of intervertebral disk between the first and second vertebrae (T1/2). Squares with CD, EF and GF in panels A and B are shown below at higher magnification. ltSCA, left subclavian artery. A smooth muscle bundle sometimes appears to be located adjacent to a striated muscle bundle (arrows in panels C–F). Note flat SMA-positive cells associated with or surrounding striated muscles (arrows in panels E and G). asterisks, intermuscular nerve elements: see Fig. 4 (PGP9.5 and S100 protein immunohistochemistry).

as a positive control for SMA immunohistochemistry (Figs. 3A and 4A), was seen running longitudinally in the cervical esophagus immediately inferior to the cricoid cartilage as well as in the thoracic esophagus. However, the muscularis mucosae was negative for desmin (Figs. 4CH).

The lower thoracic and abdominal esophagus displayed no immunorectivity for MyH, whereas no smooth muscle was identified at the level of the cricoid cartilage. Although vascular smooth muscle had not yet developed, the vascular endothelium was strongly immunopositive for SMA (see Section 2). The longus colli muscle, intercostal muscles and myocardium were helpful as positive controls for MyH immunohistochemistry (Figs. 2B and 3B). Additional observations of the entire fetal esophagus and other parts of the body are summarized in Table 1, although the present text will concentrate on the upper- and mid-thoracic esophagus. Notably, desmin was positive in both striated and smooth muscle (except for the muscularis mucosae) of the alimentary canal, including the esophagus.

Consequently, vertebrae were not useful for indicating a specific site in the esophagus at 9–16 weeks because of changes in the topographical relationship between them. Notably, however, at all stages examined, the transition zone between the striated and smooth muscle of the esophageal muscularis propria was located at levels where the esophagus was attached to the middle one-third of the trachea.

3.2. Histology of the transition zone between striated and smooth muscle in the fetal esophagus

The MyH-positive longitudinal muscle fibers were surrounded by thin, flat, SMA-positive cells (Figs. 3EG and 4FG). The density of

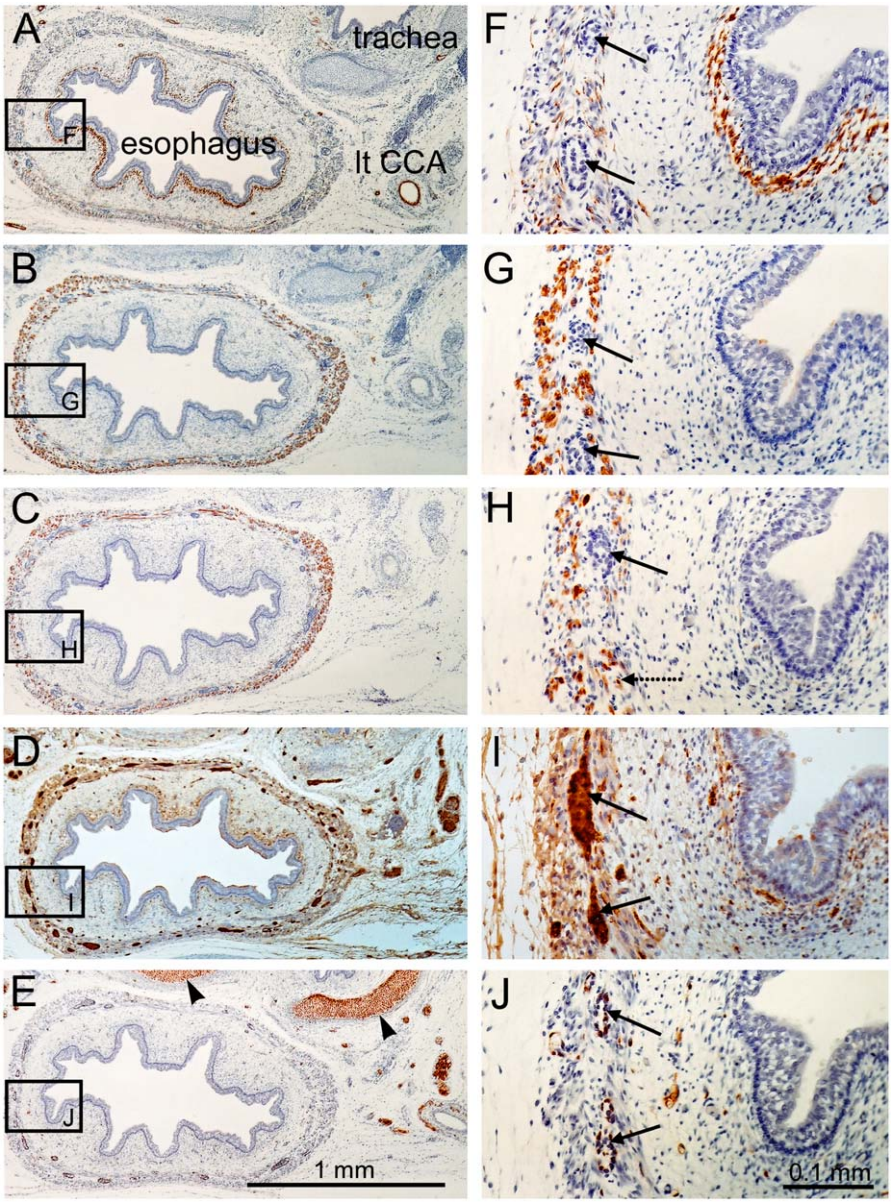

Fig. 4. S100 protein- and PGP9.5-immunohistochemistry of the esophagus in a fetus at 15 weeks of gestation. A specimen different from that shown in Fig. 3. The uppermost thoracic esophagus near the upper border of the transition zone. Panels A, B, C, D and E are SMA-, MyH-, desmin-, PGP9.5- and S100 protein immunohistochemistry, respectively. Higher magnification views of the left-hand side (square) of these panels are shown in panel F, G, H, I and J, respectively. The higher magnification views contain at least 2 intermuscular nerve elements (arrows), but panel H misses the dorsal one of them because these panels are not serial sections. The tracheal cartilage is also S100 protein-positive (arrowheads in panel E). In panels C and H, in contrast to Fig. 1B, desmin immunoreactivity is seen not only in the internal circular layer but in the external longitudinal layer. Note spindle-like SMA-positive cells associated with striated muscles (panels F and G). ltCCA, left common carotid artery.

the striated fibers was high at 2 or 3 sites per cross-section of the esophagus, particularly at the 0, 3 and 8 o'clock positions, where the external longitudinal layer was thick (Fig. 3B). In the circular layer, MyH-positive circular muscle fibers formed small clusters (Fig. 3D), which appeared to be surrounded by flat or spindle-shaped smooth muscle cells. However, SMA immunoreactivity in the circular layer was consistently weaker than in the muscularis mucosae at all sites (even in the abdominal esophagus; figures not shown). The striated muscle fibers were often or sometimes located adjacent to the SMA-positive fibers although a possibility of the double positive fibers was not denied in the present observations (Figs. 3EF).

S100-positive cells were seen in the intermuscular layer and formed clusters, but they were much more weakly stained than the vagus nerve running along the esophagus (Fig. 4E). Because the surfaces of the clusters were stained deeply with hematoxylin counterstain, they exhibited a vessel-like appearance (Figs. 3E–H and 4F–H), in contrast to the homogeneous reaction revealed by PGP9.5-immunohistochemistry (Fig. 4I). The intermuscular nerve elements tended to be rich at specific sites where the external longitudinal layer was thick (see the above paragraph). Notably, the amount of the intermuscular nerve elements did not vary between sites: i.e., above and below the transition zone. In contrast to S100- and PGP9.5-positive thick nerves, c-kit-positive cells had formed a meshwork by 12 weeks and were interdigitated with striated muscle as well as smooth muscle (Figs. 2CD). The density appeared to depend on the thickness of the esophageal wall: a thick wall carried a dense meshwork of c-kit-positive cells. Developing cartilages of the trachea and bronchi were also positive for S100. Neither CD68-

Table 1
Summary of immunohistochemistry for cytoskeleton filaments of human fetal structures at mid-term.

	Myosin heavy chain	Desmin	Smooth muscle actin
Cervical esophagus[a]	++	++	–
Mid-thoracic esophagus	++	++	+
External longitudinal	+	+	++
Internal circular	+	+	++
Muscularis mucosae	–	– or very weak	++
Abdominal esophagus[a]	–	+	++
Stomach[a]	–	+	++
Duodenum, small intestine[a]	–	+	+
Colon[a]	–	weak in circular, no in longitudinal	+
Heart[b]	++	+	– (most parts)
Trachea and bronchi[c]	–	+	++
Arterial and venous walls	–	–	++
Pharyngeal constrictors	++	++	–

[a] Muscularis propria.
[b] Areas including roots of the great vessels.
[c] Membranous part of the trachea and thick bronchi.

positive macrophages nor TUNEL-positive cells were present in the esophagus.

3.3. Histology of the adult esophagus

In the upper and mid-thoracic esophagus, SMA immunoreactivity was clearly identified. Smooth muscle fibers were intermingled with striated fibers in the internal circular layer, whereas in the external longitudinal layer, they tended to form several clusters separated clearly from striated muscle (Fig. 5). No specific localization was evident in the distribution of these clusters in cross-sections. The overall density of smooth muscle fibers was higher in the internal circular layer than the external longitudinal layer. However, the smooth muscle content varied significantly between individuals (and not between the upper and mid-thoracic levels). In 4 of 10 specimens, at the upper thoracic level, some or most of the thick circular muscle bundles were composed of smooth muscle (Fig. 5A). However, in the other 6 specimens the upper thoracic esophagus contained spindle-shaped, thin smooth muscle cells in the connective tissue bundles (Fig. 5B). The muscularis mucosae was strongly or weakly immunopositive for SMA, depending on individuals.

CD68-positive macrophages were rarely seen in the muscle layer, but were often present around glands and in the submucosal layer. The pattern of immunostaining for S100 was similar to those in the fetus, but the staining was more intense than in the latter.

4. Discussion

The present study demonstrated that a transition from smooth to striated muscle occurred in the human fetal thoracic esophagus. However, in humans, it appeared that the transition stopped at the fetal stage, and that its morphology was maintained throughout life (cf. mouse esophagus, see Section 1). In humans as well as mice, the striated muscle precursor seems to migrate caudally along the smooth-muscular esophagus and internally within the esophagus. We speculated that the muscle precursor migrates from primitive pharyngeal and/or laryngeal muscles because these two muscles communicated closely with the esophagus in fetuses as well as in adults. However, it was unclear why, in humans, the descending migration stopped at the mid-thoracic level. The influence of the lung bud and developing trachea might provide a clue because, in humans, the transition zone consistently exhibited a close topographical relationship with the adult trachea as well as with the developing trachea. Conversely, however, there is no information about the detailed topohistology of the mouse fetus.

In the mouse esophagus, three possible fates of smooth muscle have been considered: (1) transdifferentiation into striated muscle; (2) striated muscle development after apoptosis of the smooth muscle; (3) becoming static and remnant in the adult esophagus (for more details, see Introduction). To our knowledge, transdifferentiation of muscle has been reported for mouse vascular smooth muscle (Wang et al., 2006) and jellyfish striated muscle (Galle et al., 2005). Because of the limitation of the method, the present observation cannot deny a possibility that transforming muscle fibers exhibited double positive reactivities for SMA and MyH. Moreover, Wörl and Neuhuber (2005) demonstrated the presence of apoptotic smooth muscle in mice in their meticulous electron microscopy study. However, Rishniw et al. (2007, 2008) recently concluded that, in mice, esophageal striated myogenesis arises from a distinct population of myogenic precursors, rather than via transdifferentiation from smooth muscle cells, and that their "false disappearance" can be explained morphometrically.

We agree with the aforementioned theory of false disappearance of smooth muscle: striated muscle would likely become very dominant in place of smooth muscle if the latter were unlikely to increase in mass in the later stages. Thus, smooth muscle seemed to simply become dispersed throughout the late-expanded striated muscle areas. However, in human adults, the content of smooth muscle varied considerably among individuals. We did not find any CD68-positive macrophages suggesting died cells or TUNEL-positive cells in the transition zone, although the types or forms of cell death vary between tissues (Clarke, 1990) and non-apoptotic cell death is known to occur (Castro-Obregón et al., 2002). Flat smooth muscle cells were accompanied by, or intermingled with striated muscle in the adult esophagus as well as in the fetal transition zone. Therefore, we consider that the remnant smooth muscle theory is plausible. However, we do not agree with the further conclusion of Rishniw et al. (2007) that, in mice, the large majority of smooth muscle is compacted distally to give rise to the lower esophageal sphincter. Conversely, the indistinct lower sphincter in humans might result from incomplete transition of smooth to striated muscle.

Smooth muscle myosin heavy chain is exclusively restricted to smooth muscle cells with the exception of transient expression in the developing heart atrium (Regan et al., 2000; Xin et al., 2002). Because of its strong reaction in paraffin sections of long-preserved human specimens, we chose Dako alpha SMA antibody. This antibody cross-reacts with vascular endothelium (see Section 2) in addition to giving a reasonable reaction with vascular and

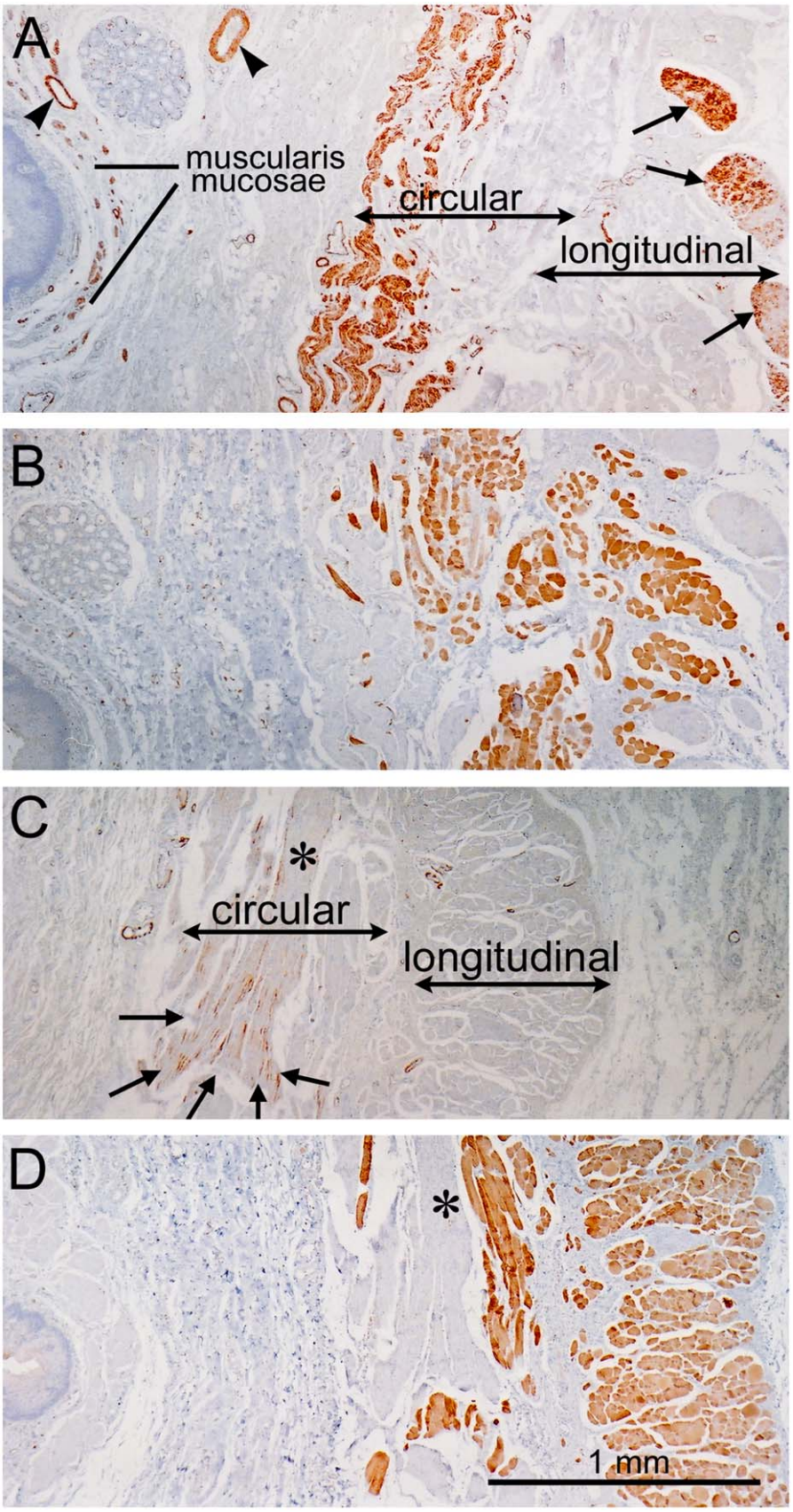

Fig. 5. Adult upper thoracic esophagus immediately superior to the aortic arch. Immunohistochemistry for SMA (panels A and C) and MyH (panels B and D), at a level immediately superior to the aortic arch. Panel A and B (C and D) display a specimen obtained from a 68 (75) year-old-man. In panel A, an internal half of the circular muscle layer is SMA-positive, whereas thick bundles or clusters (arrows) are positive in the longitudinal muscle layer. In contrast, SMA-positive cells are limited to spindle-shaped, thin muscles in panel C (arrows). A thick bundle in the circular layer (asterisk in panels C and D) was double negative for SMA and MyH. Vascular endothelium is also positive for the antibody (arrowheads in panel A). All panels were prepared at the same magnification (scale bar in panel D). Desmin appears to be positive for smooth and striated muscles of the esophagus.

non-vascular smooth muscle. In the present study, vascular smooth muscle was totally negative for the intermediate filament desmin.

In human fetuses, desmin was positive in both striated and smooth muscle of the alimentary canal including the esophagus. Notably, the muscularis mucosae generally displayed no or very weak immunoreactivity for desmin, in contrast to strong immunoreactivity for SMA. However, SMA immunoreactivity of the fetal alimentary circular muscle was usually weaker than that of the muscularis mucosae running longitudinally. Did this simply result from the difference in muscle fiber direction? The fetal cervical esophagus did not contain SMA-positive muscle but was filled with desmin-positive tissue. Was a remnant of smooth muscle "hidden" in the desmin-positive tissue? Did a phenotypic transformation into myofibroblasts occur in the remnant smooth muscle? Human fetal "lung" fibroblasts can be experimentally transformed into either a SMA-rich or a desmin-rich phenotype (Ichikawa et al., 2008). Desmin might provide a clue for understanding the suspected intermediate morphology between the smooth and striated muscle phenotypes in the esophagus. Alternatively, either desmin, SMA or both are often expressed in gastrointestinal stromal tumors in association with c-kit immunoreactivity (Abraham et al., 2007; Alvarado-Cabrero et al., 2007). Moreover, the c-kit-positive interstitial cells of Cajal become attached to the remnant smooth muscle in the developing mouse esophagus (Rishniw et al., 2007). Although transdifferentiation from smooth to striated muscle seems unlikely, parts of the remnant smooth muscle are likely to lose their SMA immunoreactivity and undergo phenotypic transformation. Smooth muscle and the interstitial cells of Cajal have a common progenitor (Lorincz et al., 2008) and smooth muscle can express c-kit (Mei et al., 2006). Actually, depending on individual fetuses (not on stages), the external and internal smooth muscle layers sometimes strongly expressed c-kit in the rectum (data, not shown). Further studies will be necessary to clarify the interactions occurring among SMA, desmin and c-kit during development of the alimentary canal.

Disclosures

This study does not include any potential conflicts.

References

Abraham, S.C., Krasinskas, A.M., Hofstetter, W.L., et al., 2007. Seedling mesenchymal tumors (gastrointestinal stromal tumors and leiomyomas) are common incidental tumors of the esophagogastric junction. Am. J. Surg. Pathol. 31, 1629–1635.

Alvarado-Cabrero, I., Vázquez, G., Sierra Santiesteban, F.I., et al., 2007. Clinicopathological study of 275 cases of gastrointestinal stromal tumors: the experience at 3 larger medical centers in Mexico. Ann. Diag. Pathol. 11, 39–45.

Breuer, C., Neuhuber, W.L., Wörl, J., 2004. development of neuromuscular junctions in mouse esophagus: morphology suggests a role for enteric co-innervation during maturation of vagal myoneural contacts. J. Comp. Neurol. 475, 47–69.

Castro-Obregón, S., del Rio, G., Chen, S.F., et al., 2002. A ligand-receptor pair that triggers a non-apoptotic form of programmed cell death. Cell. Death Differ. 9, 807–817.

Clarke, P.G., 1990. Developmental cell death: morphological diversity and multiple mechanisms. Anat. Embryol. 181, 195–213.

DeNardi, F.G., Riddell, R.H., 1997. Esophagus. In: Sternberg, S.S. (Ed.), Histology for Pathologists second ed. Lippincott-Raven, Philadelphia, pp. 461.

Dundr, P., Povysil, C., Tvrdík, D., 2009. Actin expression in neural crest cell-derived tumors including schwannomas, malignant peripheral nerve sheath tumors, neurofibromas and melanocytic tumors. Pathol Int. 59, 86–90.

Farah-Klibi, F., Ben Hamouda, S., Ben Romdhane, S., et al., 2008. Immunohistochemical study of endometrial stromal sarcoma and smooth-muscle tumors of the uterus. J. Gynecol. Obstet. Biol. Reprod. 37, 457–762.

Galle, S., Yanze, N., Seipel, K., 2005. The homeobox gene Msx in development and transdifferentiation of jellyfish striated muscle. Int. J. Dev. Biol. 49, 961–967.

Gerecht-Nir, S., Osenberg, S., Nevo, O., et al., 2004. Vascular development in early human embryos and in teratomas derived from human embryonic stem cells. Biol. Reprod. 71, 2029–2036.

Goyal, R.K., Chaudhury, A., 2008. Physiology of normal esophageal motility. J. Clin. Gastroenterol. 42, 610–619.

Hayashi, S., Murakami, G., Ohtsuka, A., et al., 2008. Connective tissue configuration in the human liver hilar region with special reference to the liver capsule and vascular sheath. J. Hepatobiliary Pancreat. Surg. 15, 640–647.

Ichikawa, T., Sugihara, H., Koarai, A., et al., 2008. Peroxynitrate auguments fibroblast-mediated tissue remodeling via myofibroblast differentiation. Am. J. Physiol. Lung Mol. Physiol. 295, L800–0.

Kabler, B., Tajbakhsh, S., Rudnicki, M.A., 2000. Transdifferentiation of esophagus smooth muscle to skeletal muscle is myogenic bHLH factor-dependent. Development 127, 1627–1639.

Kreplak, L., Fudge, D., 2007. Biomechanical properties of intermediate filaments: from tissues to single filaments and back. Bioessay 29, 26–35.

Lorincz, A., Redelman, D., Horváth, V.J., et al., 2008. Progenitors of interstitial cells of Cajal in the postnatal murine stomach. Gastroenterology 134, 1083–1093.

Mei, F., Yu, B., Ma, H., et al., 2006. Interstitial cells of Cajal could regenerate and restore their normal distribution after disrupted by intestinal transaction and anastomosis in the adult guinea pigs. Virchows Arch. 449, 348–357.

Patapoutian, A., Wold, B.J., Wagner, R.A., 1995. Evidence for developmentally programmed transdifferentiation in mouse esophagus muscle. Science 270 (5243), 1818–1821.

Reddy, T., Kabler, B., 2004. Evidence for the involvement of neurotrophins in muscle transdifferentiation and acetylcholine receptor transformation in the esophagus of Myf5(-/-):MyoD8(-/-) and NT-3(-/-) embryos. Dev. Dyn. 231, 683–692.

Regan, C.P., Manabe, I., Owens, G.K., 2000. Development of a smooth muscle-targeted crc recombinase mouse reveals novel insights regarding smooth muscle myosin heavy chain promoter regulation. Circ. Res. 87, 363–369.

Rishniw, M., Fisher, P.W., Doran, R.M., et al., 2007. Smooth muscle persists in the muscularis externa of developing and adult mouse esophagus. J. Muscle Res. Cell Motil. 28, 153–165.

Rishniw, M., Fisher, P.J., Doran, R.M., et al., 2008. Striated myogenesis and peristalsis in the fetal murine esophagus occur without cell migration or interstitial cells of Cajal. Cells Tissues Organs 173, 1–6.

Sang, Q., Young, H.M., 1997. Development of nicotinic receptor clusters and innervations accompanying the change in muscle phenotype in the mouse esophagus. J. Comp. Neurol. 386, 119–136.

Standring, S., 2005. Gray's Anatomy, 986–990. Elsevier Churchill Livingstone, London 1251–1252.

Rishniw, M., Xin, H.B., Deng, K.Y., et al., 2003. Skeletal myogenesis in the mouse esophagus does not occur through transdifferentiation. Genesis 36, 81.

Stratton, C.J., Bayguinov, Y., Sanders, K.M., et al., 2000. Ultrastructural analysis of the transdifferentiation of smooth muscle to skeletal muscle in the murine esophagus. Cell Tissue Res. 301, 283–298.

Tai, W.C., Chuah, S.K., Lin, J.W., et al., 2008. Colorectal mesenchymal tumors—from smooth muscle tumors to stromal tumors. Oncol. Rep. 20, 1157–1164.

Tang, D.D., 2008. Intermediate filaments in smooth muscle. Am. J. Physiol. Cell Physiol. 294, C869–0.

Wang, H., Yan, S., Chai, H., et al., 2006. Share stress induces endothelial transdifferentiation from mouse smooth muscle cells. Biochem. Biophys. Res. Commun. 346, 860–865.

Wörl, J., Neuhuber, W.L., 2000. Spatial and temporal organization of TrkB expression in the developing musculature of the mouse esophagus. Histochem. Cell Biol. 114, 229–238.

Wörl, J., Neuhuber, W.L., 2005. Ultrastructural analysis of the smooth-to-striated transition zone in the developing mouse esophagus: emphasis on apoptosis of smooth and origin and differentiation of striated muscle cells. Dev. Dyn. 233, 964–982.

Xin, H.B., Deng, K.Y., Rishniw, M., et al., 2002. Smooth muscle expression of Cre recombinase and eGFP in transgenic mice. Physiol. Genom. 10, 211–215.

Xueyong, L., Shaozong, C., Wangzhou, L., et al., 2008. Differentiation of pericyte in wound healing: the precursor, the process, and the role of vascular endothelial cells. Wound Repair Regen. 16, 346–355.

Yajin, S., Murakami, G., Takeuchi, H., et al., 2009. The normal configuration and interindividual differences in intramural lymphatic vessels of the esophagus. J. Thoracic Cardiovasc. Surg. 137, 1–6.

Zhao, W., Dhoot, G.K., 2000a. Both smooth and skeletal muscle precursors are present in foetal mouse oesophagus and they follow different differentiation pathways. Dev. Dyn. 218, 587–602.

Zhao, W., Dhoot, G.K., 2000b. Skeletal muscle precursors in mouse esophagus are determined during early fetal development. Dev. Dyn. 219, 10–20.