Immunohistochemical Localization of Cytokeratins in the Junctional Region of Ectoderm and Endoderm

AKIHIRO HOSOYA,[1,2] SUNGWOOK KWAK,[1] EUN-JUNG KIM,[1] DECLAN PATRICK LUNNY,[3,4] E. BIRGITTE LANE,[3,4] SUNG-WON CHO,[1] AND HAN-SUNG JUNG[1]*

[1]Division in Anatomy and Developmental Biology, Department of Oral Biology, Research Center for Orofacial Hard Tissue Regeneration, Brain Korea 21 Project, Yonsei University College of Dentistry, Seoul, Republic of Korea
[2]Department of Oral Histology, Matsumoto Dental University, Nagano, Japan
[3]Centre for Molecular Medicine, Singapore, Singapore
[4]Cancer Research UK Cell Structure Research Group, School of Life Sciences, University of Dundee, Dundee, United Kingdom

ABSTRACT

Although tridermic species have two junctional regions of ectoderm and endoderm between their epidermis and digestive tract, we actually know little about these particular boundaries. Cytokeratins are the major intermediate filaments of epithelial cells and show a high degree of tissue specificity. Therefore, to characterize the epithelial cells in the junctional region of ectoderm and endoderm, we immunohistochemically examined the localization of cytokeratins 5, 7/17, 14, 18, Sox17, and alpha-fetoprotein (AFP) in the oropharyngeal and anorectal regions during the mouse gastrulation process. At embryonic day (E) 9.5, cytokeratins 5, 7/17, 14, and 18 were detected in all epithelial cells of the oropharyngeal region. At E12.5, cytokeratin 5-positive cells were not observed in the middle area of the oral cavity; however, the immunoreactivity was strong in the anterior and posterior areas. The immunoreaction of cytokeratins 18 was seen only in the middle and posterior areas of the oral mucosa. Cytokeratins 7/17 and 14 were localized in all areas of the oropharyngeal region. Sox17 and AFP, which are endodermal markers, were detected in the middle and posterior areas of the oral mucosa, but not in the anterior area. Moreover, this same localization pattern of cytokeratins also existed in the anorectal region of the E12.5 embryo, suggesting that the localization of cytokeratins and endodermal markers might give an implication for the boundary between ectoderm and endoderm. These results also suggest that these cytokeratins are useful molecules for monitoring the epithelial cell differentiation in the junctional region of the germ layers. Anat Rec, 293:1864–1872, 2010. © 2010 Wiley-Liss, Inc.

Key words: cytokeratin; epithelial cell; differentiation; gastrulation; ectoderm; endoderm

Grant sponsor: Seoul R&BD Program; Grant number: NT070139.

*Correspondence to: Han-Sung Jung, Ph.D., Division in Anatomy and Developmental Biology, Department of Oral Biology, Research Center for Orofacial Hard Tissue Regeneration, Brain Korea 21 Project, Yonsei University College of Dentistry, 134 Shinchon-dong, Seodaemun-gu, Seoul 120-752, Republic of Korea. Fax: +82-2-312-8012. E-mail: hsjung@yuhs.ac

Received 22 July 2009; Accepted 16 June 2010

DOI 10.1002/ar.21233

Published online 3 September 2010 in Wiley Online Library (wileyonlinelibrary.com).

INTRODUCTION

The differentiation of cells into the three germ layers including ectoderm, mesoderm, and endoderm is an important event for morphogenesis. These germ layers are formed during gastrulation, following epithelial invagination into the primitive streak. In this process, the outer layer of the embryo becomes the ectoderm; and the invaginated epithelium forming the primitive gut, which contributes to the formation of the digestive tract, liver, pancreas, and their associated organs, becomes the endoderm (Gilbert, 2006).

During the early embryogenesis of vertebrate, ectoderm and endoderm are regionalized into the organ-specific epithelia by differentiation factors emanating from the mesoderm. After that, functional organs are formed by reciprocal interactions occurring between the regionalized epithelia and their underlying mesenchyme. Therefore, the elucidation of the differentiation processes in ectoderm and endoderm development and investigation into the origin of each digestive organ might help us to obtain not only a better understanding of developmental biology but also a basic knowledge for tissue engineering therapy of ectodermal and endodermal organs.

There are two junctional regions between ectoderm and endoderm in the adult digestive organ. One is the oropharyngeal region, which becomes the entrance of the digestive tract. At early embryogenesis, the ectodermal epithelium of the embryonic head invaginates and forms the stomatodeum. The stomatodeum makes contact with the extreme anterior of the foregut to become covered with endodermal epithelium, and the oropharyngeal membrane is formed between the stomatodeum and primitive gut. After rupture of the oropharyngeal membrane, these tissues are connected; and the primary oral cavity is formed above this membrane. Thus, epithelial cells of the oral cavity, tooth enamel, and parotid gland are thought to be of ectodermal origin (Nanci, 2003); although these tissues are appendages of the digestive tract. However, since little is known of the ectodermal and endodermal cell migration during gastrulation, the regional specification of these cells is poorly understood. Another junction exists at the anorectal region, which becomes the lowest part of the digestive tract. This region is formed after cloacal opening that connects the primitive gut epithelium with the ectodermal skin. At the early stage of cloacal development, the cloaca consists of the hind gut and urogenital sinus; whereas this cavity is separated from the skin ectoderm by the cloacal membrane. Next, the cloaca invaginates into the cloacal membrane, and the endodermal urorectal septum also invaginates between the hindgut and urogenital sinus. After the disintegration of the cloacal membrane, the hindgut attaches to the ectodermal skin and is separated from the urogenital sinus by the endodermal urorectal septum (de Vries and Friedland, 1974; Miller and Briglin, 1996; Sasaki et al., 2004). However, it is unclear whether the fusion of the endodermal urorectal septum with the ectodermal skin occurs during these processes. Therefore, the actual boundaries between ectoderm and endoderm in adult tissues are unclear.

Recently, many genes have been reported to be expressed during early embryogenesis; and these include some specific molecules in the endoderm, such as Sox 17 and alpha-fetoprotein (AFP; Technau and Scholz, 2003; Grapin-Botton and Constam, 2007). Sox17 has been shown to be expressed specifically in the endoderm during gastrulation in Xenopus (Hudson et al., 1997; Clements and Woodland, 2000) and zebrafish (Alexander and Stainier, 1999). Because Sox17-null mice fail to form gut endoderm (Kanai-Azuma et al., 2002), this gene is known as a determinant of endodermal differentiation. In the mouse, Sox17 is expressed in the yolk sac, pancreas, and part of the primitive gut during early embryogenesis (Kanai-Azuma et al., 2002; Lioubinski et al., 2003). AFP is the most plentiful serum protein in the mouse embryo. It is synthesized by cells of the yolk sac, fetal liver, fetal gut epithelium, and visceral endoderm (Mizejewski, 1997). Therefore, Sox17 and AFP are thought to be the useful marker for detection of the endoderm. However, since the expression level of these proteins is reduced in normal tissues as embryogenesis progresses (Sasai, 2001), it is difficult to classify and define the germ layers by detecting these marker genes.

On the other hand, cytokeratins are major cytoskeletal proteins in epithelial cells. In mammalian epithelial cells, over 20 cytokeratins has been reported to exist and are divided into the basic and acidic types (Moll et al., 1982; Cooper et al., 1985; Quinlan et al., 1985). Cytokeratin 5 is a basic-type cytokeratin localized in the nonkeratinizing stratified squamous epithelium of many tissues and transitional epithelium (Quinlan et al., 1985). Cytokeratin 7 is also a basic-type cytokeratin and is found in many ductal and glandular epithelia. In normal tissues, this protein does not become localized in stratified squamous epithelium, transitional epithelium, hepatocytes, colon, or prostate (Kasper et al., 1993). On the other hand, cytokeratins 14, 17, and 18 are of the acidic type. Cytokeratin 14 is usually expressed in stratified epithelial cell types but not in simple epithelial cell types (Ouhayoun et al., 1985). Cytokeratin 17 has been found in basal cells of stratified squamous epithelium, glandular epithelium with myoepithelial components, and transitional epithelium (Troyanovsky et al., 1989). Cytokeratin 18 appears to be expressed in simple ductal and glandular epithelium, and in basal epithelial cells of stratified squamous epithelium except for the skin (Bartek et al., 1991; Kasper et al., 1993, 1994). These cytokeratins show high specificity in many tissues including the oral epithelium and epidermis (Heyden et al., 1992; Pelissier et al., 1992). Therefore, the distribution of different types of cytokeratins in the developing epithelium might be used for cell typing and identification.

In the present study, we show that cytokeratins 5, 7, 14, 17, and 18 became localized in the oropharyngeal and anorectal regions during gastrulation. In addition, to characterize the epithelial cells in the junctional region between ectoderm and endoderm, we also immunohistochemically evaluated the expression of Sox17 and AFP during this process.

MATERIALS AND METHODS

Animals

All experiments were performed according to the strict guidelines set forth by the Intramural Animal Use and Care Committee of Yonsei University College of Dentistry. Adult Institute of Cancer Research mice were housed in a temperature-controlled room (22°C) under artificial illumination (lights on from 0500 to 1700 hr) and 55% relative humidity. The mice had access to food and water *ad libitum*. Embryos were obtained from time-mated

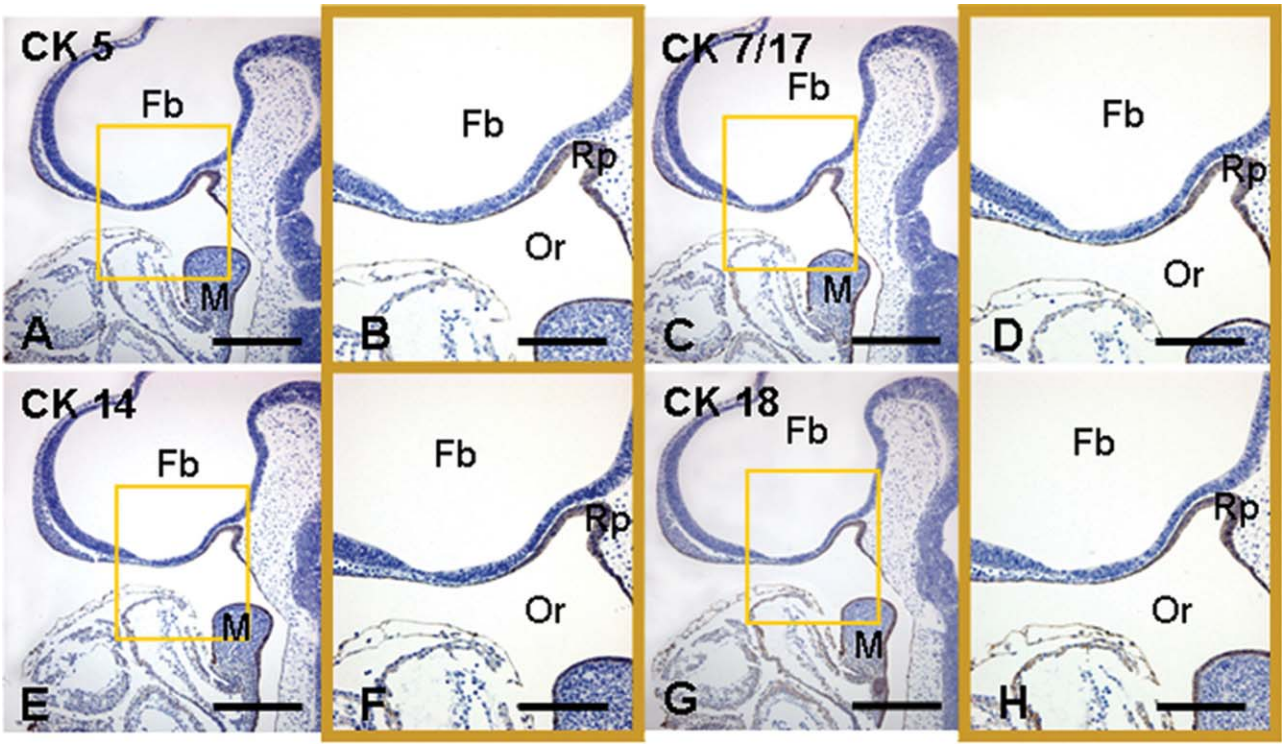

Fig. 1. Immunohistochemical staining for cytokeratins 5 (**A** and **B**), 7/17 (**C** and **D**), 14 (**E** and **F**), and 18 (**G** and **H**) in the oropharyngeal region at E9.5. Higher magnification of the boxed regions in A, C, E, and G are shown in B, D, F, and H, respectively. Cytokeratins 5, 7/17, 14, and 18 are localized in the epidermis and mucous membrane. Fb, forebrain; M, mandibular component of first branchial arch; Or, oropharynx; Rp, Rathke's pouch. Scale bars: 300 μm (A, C, E, and G), 120 μm (B, D, F, and H).

pregnant mice. Embryonic day (E) 0 was designated as the day a vaginal plug was confirmed. Embryos at developmental stages E9.5 and 12.5 were used in this study.

Immunohistochemistry

Embryos at E9.5 and 12.5 were collected under observation by stereomicroscopy. These specimens were fixed with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) at 4°C for 24 hr. After dehydration in ethanol, some samples were embedded in paraffin and sectioned sagittally at a thickness of 5 μm by using a microtome (Leica RM2165; Leica Instruments, Germany). Following deparaffinization with xylene and rehydration with ethanol, the sections were subjected to autoclaving (Cell marque, CA) while immersed in citric acid buffer (pH 6.0; Zymed Laboratories, CA), at 121°C for 15 min for antigen retrieval. After having been kept at room temperature for 20 min, they were next treated with 0.3% H_2O_2 in methanol for 15 min at room temperature to inactivate endogenous peroxidase. They were then pretreated with goat serum (Zymed Laboratories) or bovine serum albumin (Seikagaku, Tokyo, Japan) in 0.01 M phosphate-buffered saline (pH 7.2; PBS) for 20 min at room temperature and subsequently incubated with rabbit polyclonal antibody against human cytokeratin 5 (abcam, Cambridge, UK) or AFP (Dako Cytomation, Glostrup, Denmark), goat polyclonal antibody against mouse Sox17 (S-20, Santa Cruz Biotechnology, CA), mouse monoclonal antibodies against cytokeratins 7/17 (LP1K; Huilgol et al., 1998), 14 (LL001; Russell et al., 2004), or 18 (LDK18; Schutte et al., 2004; Lee et al., 2006) for 12 hr at 4°C. The antibodies against cytokeratin 5 and AFP were diluted to 1:1000; and cytokeratin 7, 14, 18, and Sox17 antibodies, to 1:100. Next, the sections were rinsed in PBS and reacted with biotinylated goat antibody against rabbit IgG (Zymed Laboratories), goat antibody against mouse IgG (Dako Cytomation, CA), or rabbit antibody against goat IgG (Zymed Laboratories) for 10 min at room temperature. They were then reacted with horseradish peroxidase (HRP)-conjugated streptavidin (Zymed Laboratories). After a PBS wash, the immune complexes were visualized by using 3,3'-diaminobenzidine tetrahydrochloride (Liquid DAB Substrate kit; Zymed Laboratories). Immunostained sections were counter-stained with Mayer's hematoxylin (Lab Vision, CA). Non-immune mouse or rabbit sera were diluted to the same strength for use as negative controls. Control sections did not show any specific immunoreactivity.

RESULTS

Localization of Cytokeratins in the Oropharyngeal Region of the E9.5 Mouse Embryo

In this stage, the oropharyngeal membrane had already disappeared, and the stomatodeum and primitive gut had made the oropharynx (Fig. 1A,C,E,G). Epithelial cells of this region were classified as stratified and simple epithelium. Cytokeratins 5, 7/17, 14, and 18 were localized in the epithelial cells of the oropharynx (Fig.

1B,D,F,H). These immunoreactivities were also seen in epithelial cells of the epidermis (Fig. 1A,C,E,G). The staining patterns of cytokeratins 5, 7/17, 14, and 18 were almost the same in both of these epithelia.

Localization of Cytokeratins in the Oropharyngeal Region of the E12.5 Mouse Embryo

By E12.5, the epithelial layer of the oral cavity had divided into three parts according to epithelial morphology. The epithelium in the anterior area of the oral cavity was a stratified epithelium that shifted to the epidermis. In the middle area of the oral cavity, the epithelial layer changed to a simple epithelium and the cells showed a flat shape. The posterior oral mucosa was classified as a stratified epithelium with a squamous superficial cell layer. Cytokeratin 5 was detected in the epidermal cells and oral mucosa cells of the anterior area (Fig. 2A,B). The oral mucosa composed of flattened cells in the middle area was negative for cytokeratin 5, whereas the posterior oral mucosa showed positive immunoreactivity as the epithelial cells became multilayered (Fig. 2B,C). Cytokeratin 7/17 was localized heavily in all oral mucosa cells of the middle and posterior areas (Fig. 2D,F). In the anterior area, this immunoreactivity was detected in the superficial layer of the oral mucosa, but not in the other layers (Fig. 2E). Intense immunostaining for cytokeratin 14 was observed in the epidermal epithelium and the anterior oral mucosa, but it became gradually weaker in the oral mucosa of the middle and posterior areas (Fig. 2G–I). Epithelial cells and the anterior oral mucosa showed no immunoreactivity for cytokeratin 18 (Fig. 2J,K). However, this immunoreactivity appeared in the oral mucosa of the middle and posterior areas (Fig. 2K,L).

Localization of Cytokeratins in the Anorectal Region of the E12.5 Mouse Embryo

In the anorectal region at E12.5, the hindgut did not yet open to the outside of the body; and there were many epithelial cells between the hindgut and skin. Some cells in this cloacal membrane between the hindgut and skin showed immunoreactivity for cytokeratin 5. These positive cells were confined to the area around the pre-anal orifice. There was no evidence of the localization of cytokeratin 5 at the hindgut side of the cloacal membrane, but other hindgut mucosa and epidermal epithelium showed this immunoreactivity (Fig. 3A,B). Intense immunostaining for cytokeratin 7/17 was observed in epidermal cells, as well as in mucosa cells, of the anorectal region (Fig. 3C,D). Cytokeratin 14 demonstrated a similar pattern of the immunoreactivity as cytokeratin 7/17; however, weaker staining of the epithelium was observed (Fig. 3E,F). Cytokeratin 18 was localized in mucosa cells of the anorectal region, but not in the epidermal epithelium (Fig. 3G,H).

Localization of Endodermal Marker Proteins in the Oropharyngeal and Anorectal Regions of E12.5 Mouse

Immunoreactivity for Sox17 was not localized in the anterior oral mucosa (Fig. 4A,B). However, a nuclear labeling pattern for Sox17 was seen in epithelial cells at the middle and posterior areas of the oral mucosa (Fig. 4B,C). AFP demonstrated a similar pattern of the immunoreactivity as Sox17, but the edge of the positive area shifted a slight distance backward. Label indicating this protein was mainly detected in the cytoplasm (Fig. 4D–F). In the anorectal region, Sox17 (not shown) and AFP were hardly detected in mucosa cells or in the epidermal epithelium. These immunoreactivities were also not visible in the mesenchymal tissue of this area (Fig. 4G,H).

DISCUSSION

In the present study, the epithelial morphology in the junctional regions between ectoderm and endoderm changed from E9.5 to E12.5 in the mouse embryo; and cytokeratins 5, 7/17, 14, and 18 showed various localization patterns during the gastrulation process. These localization patterns were evident, and each cytokeratin demonstrated a different pattern. Thus, the observation of cytokeratin localization is a reliable method to characterize the epithelial cells during the gastrulation process.

Most cytokeratin fibers are tetrameric structures composed of basic and acidic types of cytokeratins in equal parts. Likewise, it is known that cytokeratins 5 and 14 are normally expressed as a pair in stratified squamous epithelial cells (Cooper et al., 1985; Tabata et al., 1996). In the present study, the epithelium of the oropharyngeal region at E9.5 showed positive immunoreactivity for cytokeratins 5 and 14. However, in the oral cavity of the E12.5 mouse embryo, cytokeratin 5-positive cells were not detected in the middle area of the oral mucosa, which was classified as a simple epithelium; even though cytokeratin 14 showed positive immunoreactivity in this area. After birth, all parts of the oral epithelial membrane become stratified squamous epithelium and show immunoreactivity for both cytokeratins (Moll et al., 1982; Ouhayoun et al., 1985). In addition, it has been reported that the expression of cytokeratin 5 and 14 changes during the epithelial cell differentiation process (Hudson et al., 2001; Hosoya et al., 2008). Therefore, the cytokeratin 5-negative and cytokeratin 14-positive region of the oral epithelium is thought to be immature, suggesting that these transitional patterns of cytokeratins 5 and 14 might be a useful marker for differentiation of oral mucosa cells in research on oral development and regeneration.

At the beginning of gastrulation, the ectodermal stomatodeum invaginates and makes contact with the endodermal foregut. As these two tissues are separated by the oropharyngeal membrane, this membrane is thought to be the junction between the ectoderm and endoderm embryologically. Rathke's pouch is located just anterior to the oropharyngeal membrane and later forms the adenohypophysis. Therefore, in adult tissue, the adenohypophysis and some digestive tract appendages such as teeth and parotid gland, which are located in the anterior of the oropharyngeal membrane, are thought to be of ectodermal origin. However, because the epithelial cells of digestive tract have high ability for proliferation, the boundary between the ectoderm and endoderm in the digestive tract may be shifted during the gastrulation process. In the present study, cytokeratin 18 showed no immunoreactivity in the anterior region of oral cavity at E12.5. Immunostaining for

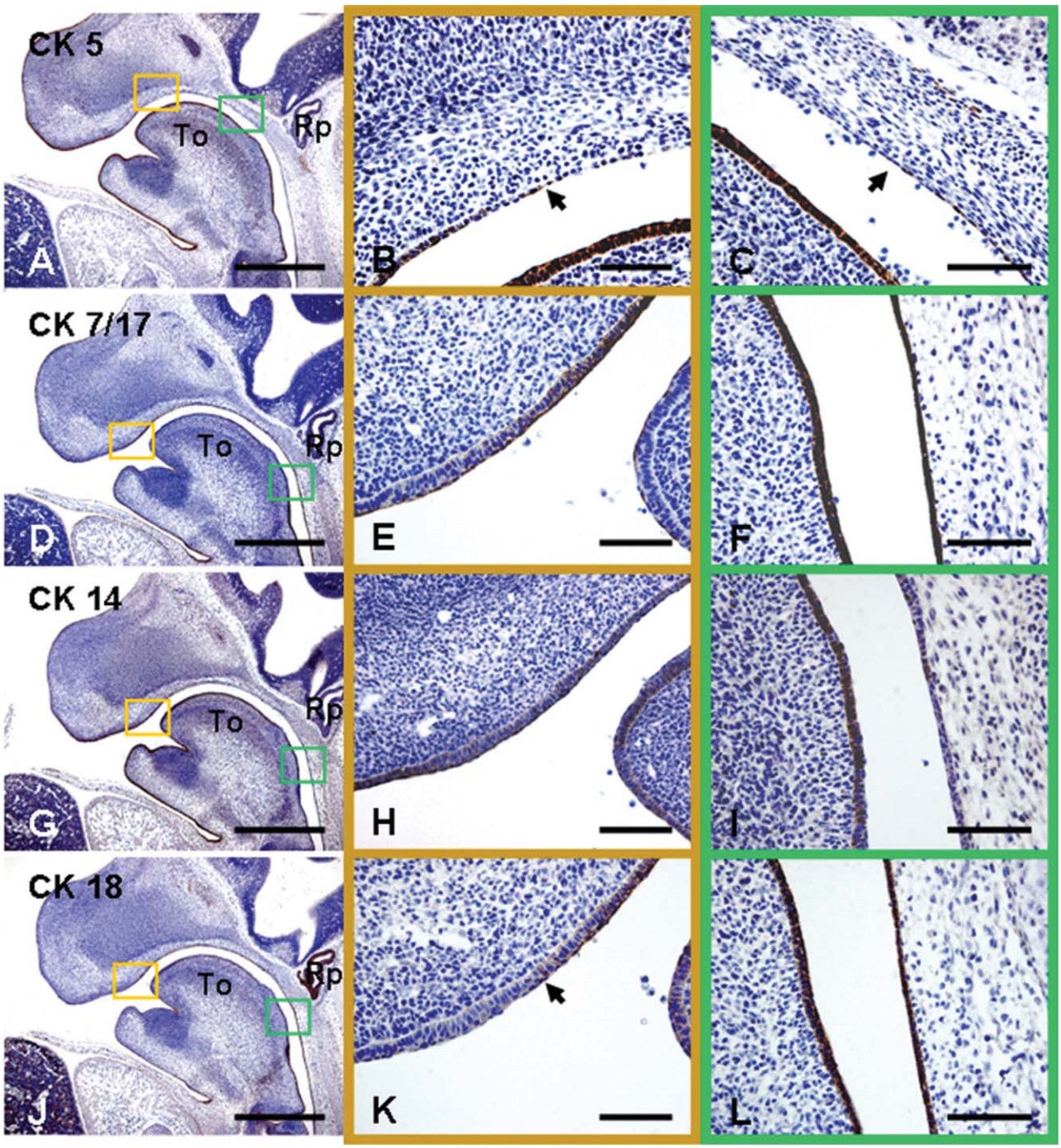

Fig. 2. Immunohistochemical stainings for cytokeratins 5 **(A–C),** 7/17 **(D–F),** 14 **(G–I),** and 18 **(J–L)** in the oropharyngeal region at E12.5. Higher magnification of the boxed regions in A, F, G, and J are shown in B–C, E–F, H–I, and K–L, respectively. A–C: Cytokeratin 5 is localized in the anterior area of the mucous membrane as well as in the epidermis. D–F: Heavy cytokeratin 7/17 localization is seen in the epidermis and the middle and posterior areas of the mucous membrane. In the anterior area, mucosa cells in the superficial layer show this immunoreactivity. G–I: Cytokeratin 14 immunoreactivity is apparent in the epidermis and all of the oral mucosa. J–L: There is no immunoreactivity for cytokeratin 18 in the epidermis and the anterior oral mucosa, whereas this immunoreactivity is observed in the middle and posterior areas of the oral mucosa. Arrows in B, C, H, and K show the edge between positive and negative areas. Rp, Rathke's pouch; To, tongue. Scale bars: 500 μm (A, D, G, and J), 50 μm (B–C, E–F, H–I, and K–L).

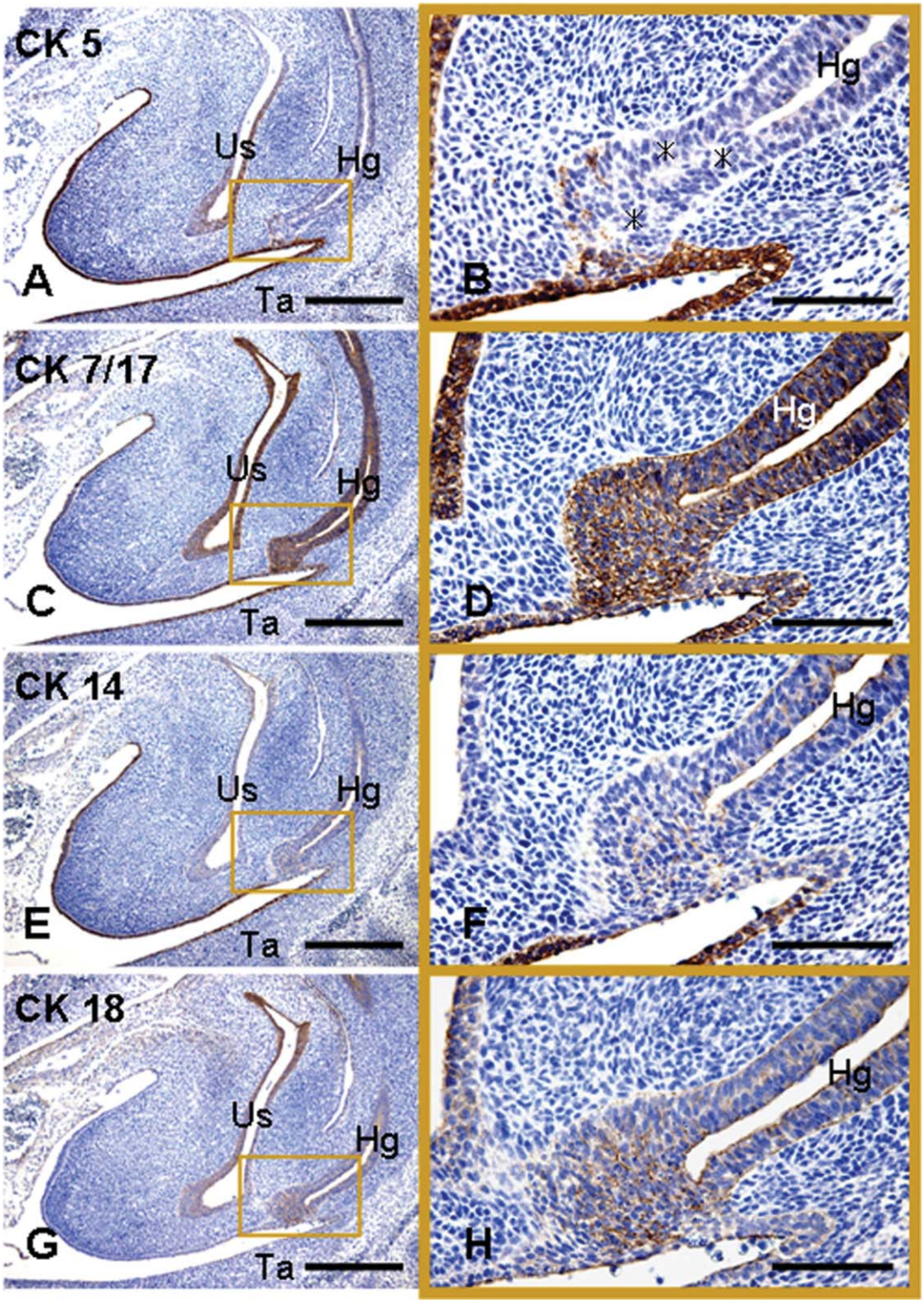

Fig. 3. Immunohistochemical staining for cytokeratins 5 **(A** and **B),** 7/17 **(C** and **D),** 14 **(E** and **F),** and 18 **(G** and **H)** in the anorectal region at E12.5. Higher magnification of the boxed regions in A, C, E, and G are shown in B, D, F, and H, respectively. A and B: Cytokeratin 5 is localized in epidermal cells and mucosal cells near the skin. These positive cells are scarce at the distal part of the hindgut (asterisks), but other hind gut mucosa shows the immunoreactivity for cytokeratin 5. C–F: Immunoreactivities for cytokeratins 7/17 and 14 are observed in the epidermis and mucosa of the hindgut (Hg). G and H: Cytokeratin 18 is detected in all mucosa cells of the hindgut, but scarcely in the epidermis. Ta, tail; Us, urogenital sinus. Scale bars: 300 μm (A, C, E, and G), 100 μm (B, D, F, and H).

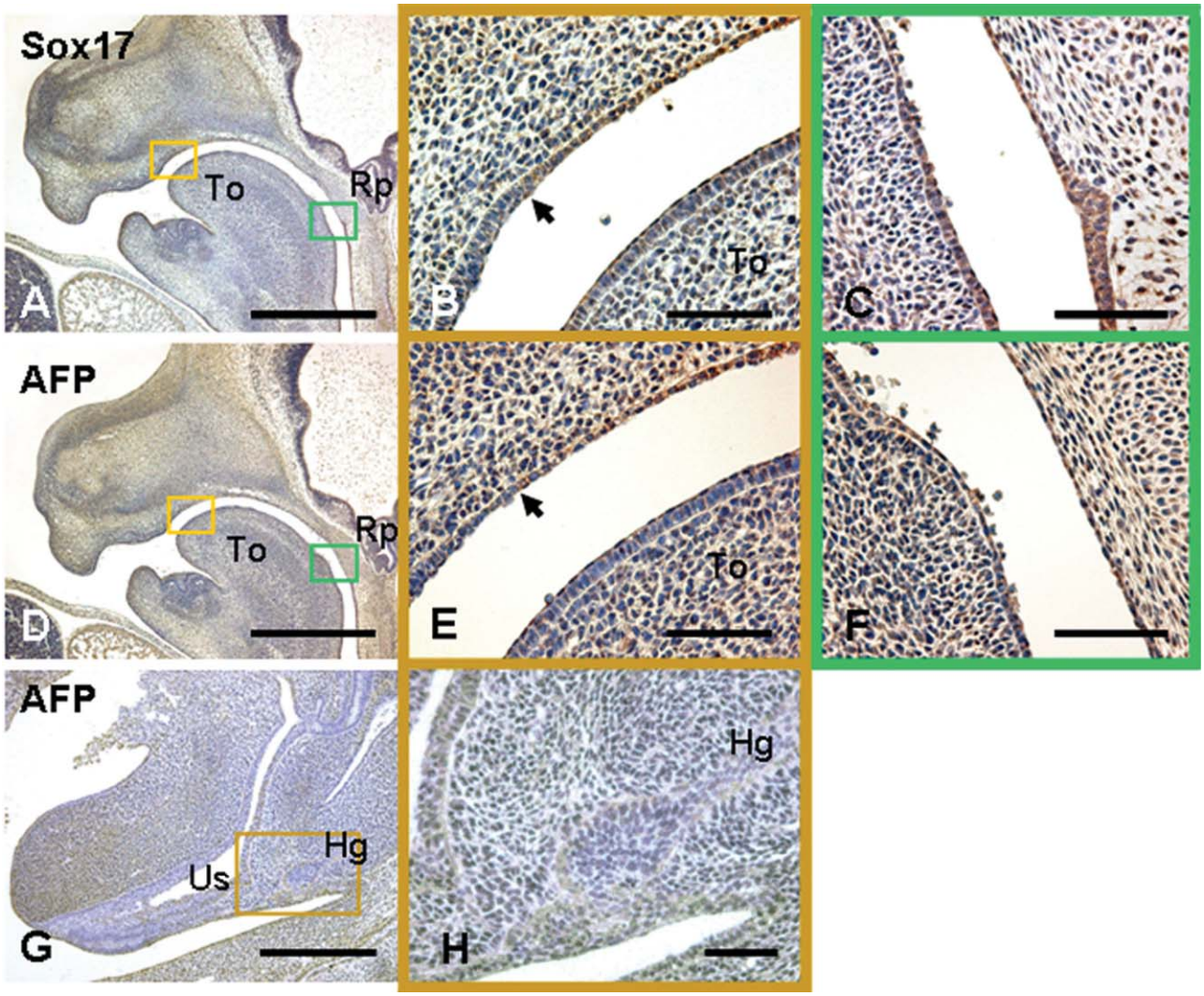

Fig. 4. Immunohistochemical staining for Sox17 **(A–C)** and alpha-fetoprotein **(D–H)** in the oropharyngeal (A–F) and the anorectal (G–H) regions at E12.5. Higher magnification of the boxed regions in A, D, and G are shown in B–C, E–F, and H, respectively. A–C: Sox17 is localized in the middle and posterior areas of the oral mucosa, but not in the anterior area. D–F: Alpha-fetoprotein (AFP) is detected in epithelial cells of the middle and posterior areas of oral cavity. G–H: AFP-positive epithelial cells are scarce in the anorectal region. Arrows in B and E show the edge between positive and negative areas. Hg, hindgut; Rp, Rathke's pouch; To, tongue; Us, urogenital sinus. Scale bars: 500 μm (A and D), 300 μm (G), 50 μm (B–C, E–F, and H).

cytokeratin 7/17 was not also observed in this region except for cells in the superficial layer. However, these immunoreactivities for cytokeratins 7/17 and 18 appeared in the middle and posterior regions and partially overlapped with cytokeratin 5- and 14-positive area. In addition, Sox17 and AFP immunoreactivities, which were observed in the middle and posterior area of the oral cavity, were disappeared in this area of overlap (Fig. 5A). As Sox17 and AFP are endodermal markers (Technau and Scholz, 2003; Fukuda and Kikuchi, 2005; Grapin-Botton and Constam, 2007), the overlap region of the immunoreactivity for cytokeratins 5, 7/17, 14, and 18 might be the boundary between the ectoderm and endoderm. We confirmed that the dental lamina existed in this overlap region by examining serial sections. Tooth development is initiated by reciprocal interactions between epithelial and mesenchymal cells (Nanci, 2003) and these mesenchymal cells are of neural crest origin (Chai et al., 2000; Yamazaki et al., 2007). Thus, we consider the dental lamina showing cytokeratins 5, 7/17, 14, and 18 immunoreactivities to be a special epithelium in the oral cavity of the E12.5 mouse embryo. In the anorectal region at E12.5, mucosa cells close to the epidermis expressed all these cytokeratins; and their relationship was consistent with that in the oropharyngeal region (Fig. 5B).

In the present study, Sox17 and AFP did not show any specific immunoreactivity in the oropharyngeal region of the E9.5 mouse embryo (not shown), in which almost all the cells are believed to be undifferentiated. In addition, in the anorectal region of the E12.5 embryo, none of the epithelial cells showed any immunohistochemical

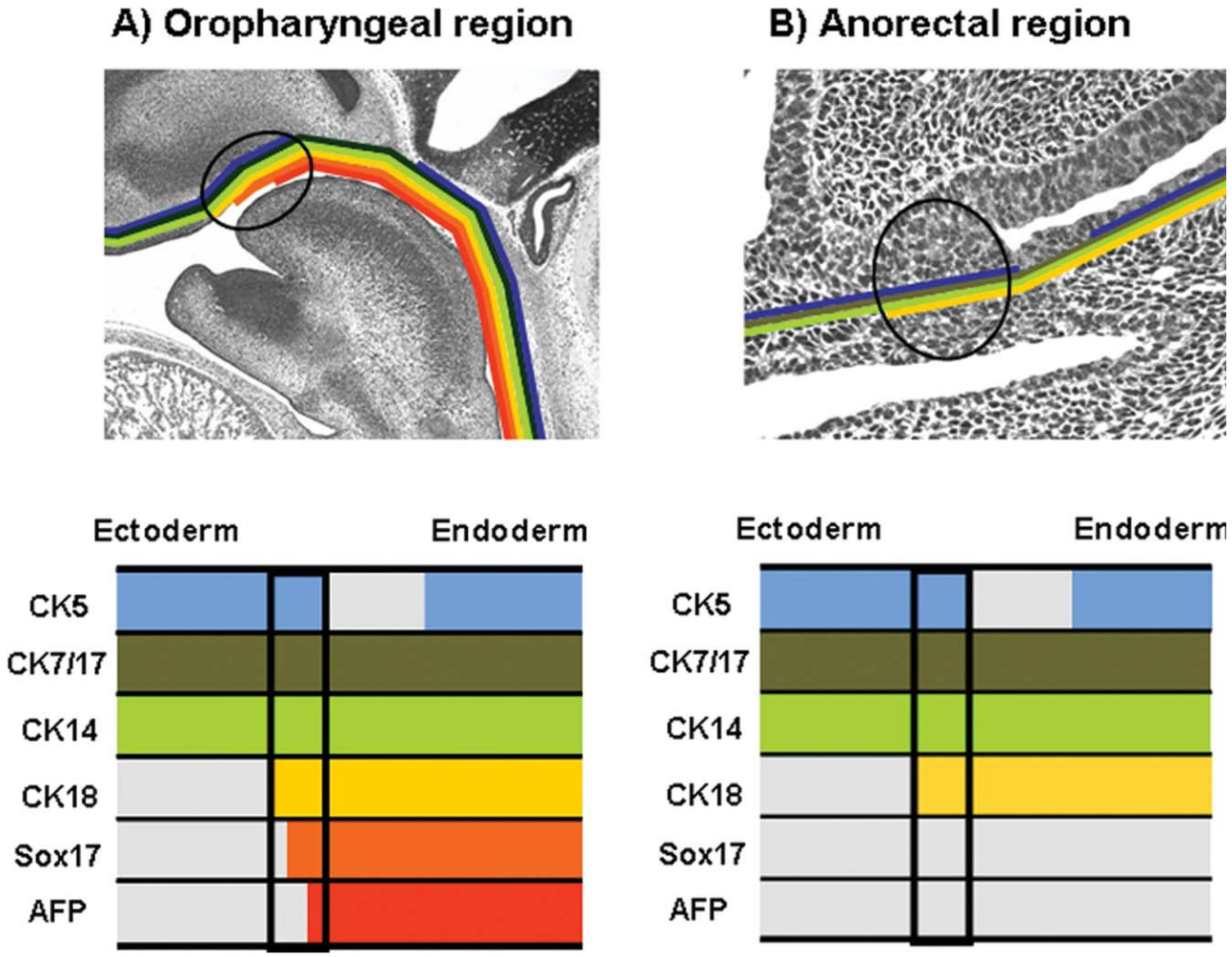

Fig. 5. Schematic illustration of immunohistochemical localization of cytokeratins and endodermal marker proteins in the oropharyngeal **(A)** and anorectal **(B)** region of the E12.5 mouse embryo. All cytokeratins show immunoreactivity at a limited area between the anterior and middle parts of the oral cavity (A) and at the pre-anal orifice (B). These areas of overlap are circled by a black line.

localizations for these endodermal markers. Therefore, because it was difficult to decide the boundary between the ectoderm and endoderm by use of Sox17- and AFP-specific antibodies, we tried to characterize the epithelial cells by using a combination of cytokeratins and endodermal markers. There are some specific ectodermal cell differentiation markers, such as Sox2 and ectodemin (Rex et al., 1997; Wood and Episkopou, 1999; Dupont et al., 2005). However, these proteins did not show definite localization histologically in the junctional region of mouse germ layers. Further research into markers of the germ layers during embryogenesis is required.

In conclusion, immunohistochemical localization of cytokeratins showed various patterns in the junctional region between the ectoderm and endoderm during the gastrulation process. This study indicates that cytokeratins are useful molecules to observe the progression of epithelial cell differentiation. Our results also suggest that this specific staining pattern of cytokeratins might show the junction between ectoderm and endoderm.

LITERATURE CITED

Alexander J, Stainier DY. 1999. A molecular pathway leading to endoderm formation in zebrafish. Curr Biol 21:1147–1157.

Barrett AW, Cort EM, Patel P, Berkovitz BK. 2000. An immunohistological study of cytokeratin 20 in human and mammalian oral epithelium. Arch Oral Biol 45:879–887.

Barrett AW, Selvarajah S, Franey S, Wills KA, Berkovitz BK. 1998. Interspecies variations in oral epithelial cytokeratin expression. J Anat 193:185–193.

Bartek J, Vojtesek B, Staskova Z, Bartkova J, Kerekes Z, Rejthar A, Kovarik J. 1991. A series of 14 new monoclonal antibodies to keratins: characterization and value in diagnostic histopathology. J Pathol 164:215–224.

Chai Y, Jiang X, Ito Y, Bringas P, Jr., Han J, Rowitch DH, Soriano P, McMahon AP, Sucov HM. 2000. Fate of the mammalian cranial neural crest during tooth and mandibular morphogenesis. Development 127:1671–1679.

Clements D, Woodland HR. 2000. Changes in embryonic cell fate produced by expression of an endodermal transcription factor, Xsox17. Mech Dev 99:65–70.

Cooper D, Schermer A, Sun TT. 1985. Classification of human epithelia and their neoplasms using monoclonal antibodies to

keratins: strategies, applications, and limitations. Lab Invest 52:243–256.
de Vries PA, Friedland GW. 1974. The staged sequential development of the anus and rectum in human embryos and fetuses. J Pediatr Surg 9:755–769.
Domingues MG, Jaeger MM, Araujo VC, Araujo NS. 2000. Expression of cytokeratins in human enamel organ. Eur J Oral Sci 108: 43–47.
Dupont S, Zacchigna L, Cordenonsi M, Soligo S, Adorno M, Rugge M, Piccolo S. 2005. Germ-layer specification and control of cell growth by Ectodermin, a Smad4 ubiquitin ligase. Cell 121:87–99.
Fukuda K, Kikuchi Y. 2005. Endoderm development in vertebrates: fate mapping, induction and regional specification. Dev Growth Differ 47:343–355.
Gao Z, Mackenzie IC, Cruchley AT, Williams DM, Leigh I, Lane EB. 1989. Cytokeratin expression of the odontogenic epithelia in dental follicles and developmental cysts. J Oral Pathol Med 18:63–67.
Gilbert SF. 2006. Developmental biology. 8th ed. MA: Sinauer Associates Inc. p 25–47.
Grapin-Botton A, Constam D. 2007. Evolution of the mechanisms and molecular control of endoderm formation. Mech Dev 124:253–278.
Heikinheimo K, Hormia M, Stenman G, Virtanen I, Happonen RP. 1989. Patterns of expression of intermediate filaments in ameloblastoma and human fetal tooth germ. J Oral Pathol Med 18:264–273.
Heyden A, Huitfeldt HS, Koppang HS, Thrane PS, Bryne M, Brandtzaeg P. 1992. Cytokeratins as epithelial differentiation markers in premalignant and malignant oral lesions. J Oral Pathol Med 21:7–11.
Hosoya A, Lee JM, Cho SW, Kim JY, Shinozaki N, Shibahara T, Shimono M, Jung HS. 2008. Morphological evidence of basal keratinocyte migration during the re-epithelialization process. Histochem Cell Biol 130:1165–1175.
Hudson C, Clements D, Friday RV, Stott D, Woodland HR. 1997. Xsox17alpha and -beta mediate endoderm formation in Xenopus. Cell 91:397–405.
Hudson DL, Guy AT, Fry P, O'Hare MJ, Watt FM, Masters JR. 2001. Epithelial cell differentiation pathways in the human prostate: identification of intermediate phenotypes by keratin expression. J Histochem Cytochem 49:271–278.
Huilgol SC, Ramnarain N, Carrington P, Leigh IM, Black MM. 1998. Cytokeratins in primary cutaneous amyloidosis. Australas J Dermatol 39:81–85.
Kanai-Azuma M, Kanai Y, Gad JM, Tajima Y, Taya C, Kurohmaru M, Sanai Y, Yonekawa H, Yazaki K, Tam PP, Hayashi Y. 2002. Depletion of definitive gut endoderm in Sox17-null mutant mice. Development 129:2367–2379.
Kasper M, Hofer D, Woodcock-Mitchell J, Migheli A, Attanasio A, Rudolf T, Muller M, Drenckhahn D. 1994. Colocalization of cytokeratin 18 and villin in type III alveolar cells (brush cells) of the rat lung. Histochemistry 101:57–62.
Kasper M, Karsten U, Stosiek P, Moll R. 1989. Distribution of intermediate-filament proteins in the human enamel organ: unusually complex pattern of coexpression of cytokeratin polypeptides and vimentin. Differentiation 40:207–214.
Kasper M, Rudolf T, Verhofstad AA, Schuh D, Muller M. 1993. Heterogeneity in the immunolocalization of cytokeratin-specific monoclonal antibodies in the rat lung: evaluation of three different alveolar epithelial cell types. Histochemistry 100:65–71.
Lee MJ, Kim JY, Lee SI, Sasaki H, Lunny DP, Lane EB, Jung HS. 2006. Association of Shh and Ptc with keratin localization in the initiation of the formation of circumvallate papilla and von Ebner's gland. Cell Tissue Res 352:253–261.
Lioubinski O, Müller M, Wegner M, Sander M. 2003. Expression of Sox transcription factors in the developing mouse pancreas. Dev Dyn 227:402–408.
Miller SA, Briglin A. 1996. Apoptosis removes chick embryo tail gut and remnant of the primitive streak. Dev Dyn 206:212–218.
Moll R, Franke WW, Schiller DL, Geiger B, Krepler R. 1982. The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cells. Cell 31:11–24.
Nanci A. 2003. Ten Cate's oral histology: development, structure, and function. 6th ed. St. Louis: Mosby, Inc. p 79–110.
Ouhayoun JP, Gosselin F, Forest N, Winter S, Franke WW. 1985. Cytokeratin patterns of human oral epithelia: differences in cytokeratin synthesis in gingival epithelium and the adjacent alveolar mucosa. Differentiation 30:123–129.
Pelissier A, Ouhayoun JP, Sawaf MH, Forest N. 1990. Evolution of cytokeratin expression in developing human tooth germ. J Biol Buccale 18:99–108.
Pelissier A, Ouhayoun JP, Sawaf MH, Forest N. 1992. Changes in cytokeratin expression during the development of the human oral mucosa. J Periodontal Res 27:588–598.
Quinlan RA, Schiller DL, Hatzfeld M, Achtstatter T, Moll R, Jorcano JL, Magin TM, Franke WW. 1985. Patterns of expression and organization of cytokeratin intermediate filaments. Ann N Y Acad Sci 455:282–306.
Rex M, Orme A, Uwanogho D, Tointon K, Wigmore PM, Sharpe PT, Scotting PJ. 1997. Dynamic expression of chicken Sox2 and Sox3 genes in ectoderm induced to form neural tissue. Dev Dyn 209: 323–332.
Russell D, Andrews PD, James J, Lane EB. 2004. Mechanical stress induces profound remodeling of keratin filaments and cell junctions in epidermolysis bullosa simplex keratinocytes. J Cell Sci 117:5233–5243.
Sasai Y. 2001. Roles of Sox factors in neural determination: conserved signaling in evolution?Int J Dev Biol 45:321–326.
Sasaki C, Yamaguchi K, Akita K. 2004. Spatiotemporal distribution of apoptosis during normal cloacal development in mice. Anat Rec A Discov Mol Cell Evol Biol 279:761–767.
Schutte B, Henfling M, Kölgen W, Bouman M, Meex S, Leers MP, Nap M, Björklund V, Björklund P, Björklund B, Lane EB, Omary MB, Jörnvall H, Ramaekers FC. 2004. Keratin 8/18 breakdown and reorganization during apoptosis. Exp Cell Res 297:11–26.
Sun TT, Tseng SC, Huang AJ, Cooper D, Schermer A, Lynch MH, Weiss R, Eichner R. 1985. Monoclonal antibody studies of mammalian epithelial keratins: a review. Ann N Y Acad Sci 455:307–329.
Tabata MJ, Matsumura T, Liu JG, Wakisaka S, Kurisu K. 1996. Expression of cytokeratin 14 in ameloblast-lineage cells of the developing tooth of rat, both in vivo and in vitro. Arch Oral Biol 41:1019–1027.
Technau U, Scholz CB. 2003. Origin and evolution of endoderm and mesoderm. Int J Dev Biol 47:531–539.
Troyanovsky SM, Guelstein VI, Tchipysheva TA, Krutovskikh VA, Bannikov GA. 1989. Patterns of expression of keratin 17 in human epithelia: dependency on cell position. J Cell Sci 93:419–426.
Wood HB, Episkopou V. 1999. Comparative expression of the mouse Sox1, Sox2 and Sox3 genes from pre-gastrulation to early somite stages. Mech Dev 86:197–201.
Yamazaki H, Tsuneto M, Yoshino M, Yamamura K, Hayashi S. 2007. Potential of dental mesenchymal cells in developing teeth. Stem Cells 25:78–87.