The Intermediate Epithelium Lining the Larynx of the *Suncus murinus*

By

Takashi NAKANO, Yoshie IWAMA, Kaori HASEGAWA and Hiroshi MUTO

Department of Anatomy, Aichi Medical University,
Nagakute, Aichi 480-11, Japan

—Received for Publication, April 27, 1989—

Key words: Larynx, Intermediate epithelium, *Suncus murinus*

Summary: In the larynx of the *Suncus murinus* the stratified squamous epithelium lines the tip of the caudal surface of the epiglottis and the region close to the vocal process of the arytenoid cartilage, while the ciliated columnar epithelium is located in the laryngeal sac and the dorsal part of the vocal fold. Further, in the transitional zone between the stratified squamous epithelium and the ciliated columnar one there exists the epithelium which shows gradations ranging from stratified squamous through stratified cuboidal to ciliated stratified low-columnar type. It is suggested that the epithelium lining the transitional zone is identical with the “intermediate epithelium” in the mouse nasopharynx and larynx.

The *Suncus murinus* (Insectivora), originated from wild populations in Japan, retains many primitive characteristics and recently has been given close attention as a new laboratory animal in Japan. It is the purpose of this paper to study the epithelium lining the *Suncus* larynx, especially the transitional zone between the stratified squamous epithelium and the ciliated columnar one.

On the other hand, it has been pointed out that the overwhelming majority of laryngeal carcinoma arises from the respiratory epithelium which has undergone squamous metaplastic changes (Ackerman and Regato, 1962; Sato, 1968; Ishida, 1972; Yamada, 1976; Kirikae, 1977). The present authors reported that the “intermediate epithelium” occupied the transitional zone between the stratified squamous epithelium and the ciliated columnar one in the mouse nasopharynx

(Nakano, 1986) and larynx (Nakano and Muto, 1988), and suggested that the epithelium showed various stages transforming from the ciliated columnar to the stratified squamous epithelium. That is, the epithelium occupying the transitional zone in the larynx as well as in the nasopharynx must be of interest, because pathologically it seems to be origin of carcinoma. This paper is expected to contribute towards a further understanding of the histology of the *Suncus*, and of the pathology of the laryngeal carcinoma.

Materials and Methods

The laryngeal regions removed from 10 male *Suncus murinus* aged from 6 to 12 months were studied. Light microscopy specimens were fixed in 10% formalin solution,

decalcified in 6% nitric acid, dehydrated in a graded ethanol series, and embedded in paraffin. They were serially cut into frontal or sagittal sections at a thickness of 5 μ m and stained with hematoxylin and eosin.

Scanning electron microscopy samples were fixed with 2.5% glutaraldehyde in phosphate buffer, postfixed with 2% osmium tetroxide, immersed in 2% tannic acid, and stained with 2% osmium tetroxide. They were dehydrated in graded ethanol. After replacement with isoamyl acetate and drying at critical point with liquid CO₂, they were coated with gold and observed under a Jeol-U3 scanning electron microscope.

Results

General findings

In the midline of the ventral wall of the *Suncus* larynx, just caudal to the base of the epiglottis, there exists a small pouch-like laryngeal sac. Two pairs of mucosal folds project rostromedially from the lateral wall of the larynx; the vestibular fold and the vocal one. The vocal fold is supposed by the vocal process of the arytenoid cartilage, and is directed ventrally to the midline of the ventral wall to meet its counterpart. In between the vestibular fold and the vocal one, the lumen slightly widens to form the laryngeal ventricle.

Laryngeal epithelium, Light microscopic findings

In this study the epithelium lining the rostral part of the larynx, including the vocal fold, will be described. The rostral surface of the epiglottis is lined with the keratinized stratified squamous epithelium continuing from the oral cavity, while the caudal surface is covered by the various types of epithelia (Fig. 1). In the tip of the caudal surface of the epiglottis, the stratified squamous epithelium with no cornified layer is present. When the

lumen is traced towards the base, the epithelium gradually changes into the 3-5 layered cuboidal type epithelium, with alternating patches of the stratified squamous epithelium (Fig. 2). The stratified cuboidal type epithelium is often ciliated. At the base of the epiglottis the ciliated cells gradually increase (Fig. 3), and in the laryngeal sac the lining epithelium is ciliated cuboidal or columnar type of 1-2 layers thick (Fig. 4). In some cases, the vestibular fold is lined in part by the stratified squamous epithelium. As to the dorsal wall of the larynx, the apex of the arytenoid cartilage is covered by the stratified cuboidal type epithelium which transforms into the ciliated columnar epithelium with passing through the ciliated stratified cuboidal type epithelium caudally, and to the stratified squamous epithelium lining the laryngopharynx dorsally.

When sectioned frontally, the similar gradations of the epithelium are seen. In the section through the laryngeal ventricle and the laryngeal sac, the epithelium is dorsally composed of 3-5 layers of cuboidal cells with round or oval nuclei. When the lumen is traced ventrally, the stratified cuboidal type epithelium without cilia transforms into the ciliated epithelium of 1-3 cells thick. In the laryngeal ventricle the superficial ciliated cells are cuboidal or low-columnar, while in the laryngeal sac the cells increase the height. In the section through the vocal fold, the dorsal part of the fold is lined with the ciliated columnar epithelium. Near the ventral tip of the vocal process of the arytenoid cartilage, the ciliated columnar epithelium gradually changes through the ciliated stratified low-columnar type and the stratified cuboidal type epithelium to the stratified squamous epithelium (Fig. 5).

That is, in the rostral part of the *Suncus* larynx, the stratified squamous epithelium lines the tip of the epiglottis and the region close to the vocal process of the arytenoid

cartilage, while the ciliated columnar epithelium is located in the laryngeal sac and the dorsal part of the vocal fold. Additionally, in the transitional zone between the stratified squamous epithelium and the ciliated columnar one there exists the various types of epithelia transforming from the stratified squamous to the ciliated columnar epithelium. For convenience' sake, the term "intermediate epithelium (Nakano, 1986)" will be used for the epithelium lining the transitional zone. The distribution of the epithelium is shown diagrammatically in figures 6-7. In short, the intermediate epithelium is divided into the stratified squamous, the stratified cuboidal and the ciliated stratified low-columnar types and, further, alternating patches and transitional forms of any types are usually observed.

Laryngeal epithelium, Scanning electron microscopic findings

The caudal surface of the epiglottis shows 2 basic surface appearances. In the tip of the epiglottis which is covered with the stratified squamous epithelium, the surface is covered by the relatively flat and polygonal cells to form the paving-stone-like appearance (Fig. 8). In the middle and the base, the superficial cells become more and more protruded. They bulge out from the surface to a varying degree and are dissected by depressions between adjacent cells, presenting the cobblestone appearance (Fig. 8, 9). Furthermore, it is possible to detect isolated cells as well as small groups of some cells whose apices are covered with numerous cilia (Fig. 9). The degree to which this region is lined with the ciliated cells varies significantly from *Suncus* to *Suncus*. Although the predominant appearance varies between individuals, the cobblestone appearance is more predominant the nearer the surface is to the base of the epiglottis. The cobblestone appearance extends to the vestibular fold. In some cases, however, the vestibular fold shows in part the paving-stone

appearance (Fig. 10).

In the laryngeal ventricle, the ciliated cells are scattered singly or in small groups among the superficial cells protruding from the surface. As the surface is traced from the ventricle to the sac, the ciliated cells become more numerous (Fig. 11).

The surface covered with cilia extends to the dorsal part of the vocal fold. When the fold is traced ventrally, the ciliated cells gradually decrease and the superficial cells without cilia become more numerous to form the cobblestone appearance. And then, there is a gradual transition from the cobblestone appearance to the paving-stone appearance (Fig. 12).

At higher magnification, the surfaces of individual superficial cells have microvilli and microridges. The microvilli are short nub-like in form and arranged irregularly. Occasionally, several microvilli are lined up closely in a row and show a tendency to form microridges (Fig. 13A). The microridges consist of fine surface ledge of about 0.1-0.2 μm in width, the same size as the diameter of the microvilli (Fig. 13B).

Discussion

It has been described that only the stratified squamous and the ciliated columnar epithelia are present in the larynx. From this and preceding studies, however, the epithelium which is neither stratified squamous nor ciliated columnar exists in the transitional zone between the stratified squamous epithelium and the ciliated columnar one. Some investigators have studied the epithelium lining the transitional zone, and given a variety of terms (Table 1). The multiplicity of the terms for the epithelium has led to confusion and, further, it is inappropriate to apply the term "transitional epithelium (Uchida, 1961; Yamada, 1976)" to any tissue other than the urinary

Table 1. Terms for the epithelium lining the transitional zone in the larynx 1

transitional epithelium	Uchida (1961)	man
	Yamada (1976)	man
stratified columnar epithelium	Copenhaver (1964)	man
	Greep (1966)	man
	Ham (1969)	man
	Bloom & Fawcett (1976)	man
	Ichikawa <i>et al.</i> (1977)	man
	Krstić (1981)	man
ciliated stratified columnar epithelium	Copenhaver (1964)	man
	Ham (1969)	man
	Bloom & Fawcett (1976)	man
nonciliated columnar epithelium	Hiraki (1976)	macaque
stratified columnar epithelium	Tucker <i>et al.</i> (1976)	rat
nonciliated pseudostratified columnar epithelium	Smith (1977)	rat
squamoid (type) epithelium	Lewis & Prentice (1980)	rat
	Lewis (1981)	rat
pseudostratified cuboidal epithelium	Lewis & Prentice (1980)	rat
low squamoid type epithelium	Lewis (1981)	rat
nonciliated pseudostratified epithelium	Nokubi (1985)	<i>Suncus</i>
intermediate epithelium	Nakano & Muto (1988)	mouse

system.

In the *Suncus*, Nokubi (1985) studied the larynx by use of SEM only, and mentioned that the "pseudostratified nonciliated epithelium" existed in the transitional zone. However, it is impossible to ascertain which an epithelium is pseudostratified or stratified by use of SEM only. Furthermore, Nokubi (1985) used the term pseudostratified nonciliated epithelium indiscriminately for 2 different epithelia; the epithelium lining the transitional zone, and the nonciliated cells in the ciliated columnar epithelium. In the present study, the epithelium occupying the transitional zone in the *Suncus* larynx showed gradually varying types of epithelia and was distinguished 3 types, the stratified squamous, the stratified cuboidal and the ciliated stratified low-columnar types, instead of a single type as described by Nokubi (1985). In the transitional zone in the mouse nasopharynx, Nakano (1986) reported the presence of the "intermediate epithelium" which showed gradations ranging from the stratified squamous to the ciliated columnar

type. Further, the intermediate epithelium was observed in the mouse arytenoid region (Nakano and Muto, 1986), epiglottis (Nakano and Muto, 1987), nasopalatine duct (Nakano *et al.*, 1988) and larynx (Nakano and Muto, 1988). The epithelium lining the transitional zone in the *Suncus* larynx appears to be identical with the intermediate epithelium.

At the scanning electron microscopic level, the intermediate epithelium in the *Suncus* larynx showed 2 basic surface appearances; the paving-stone appearance which consisted of relatively flat polygonal cells, and the cobblestone appearance where cells bulged out to varying degree from the surface. The former appearance corresponds to the stratified squamous type, and the latter to the stratified cuboidal type at the light microscopic level. Surface appearances very similar to the intermediate epithelium have been observed in the larynx of some mammals, and assigned various terms (Table 2). Considered from their appearances, it is suggested that the microvillous cell (Andrews, 1974) and transitional cell (Nokubi, 1985) correspond to the

Table 2. Terms for the epithelium lining the transitional zone in the larynx 2

microvillous cell	Andrews (1974)	rat
nonciliated cell	Hiraki (1976)	Japanese monkey
dome-like cell	Kasuya (1976)	man
microridge cell	Breipohl <i>et al.</i> (1977)	rat
nonciliated pseudostratified cell	Nokubi <i>et al.</i> (1983)	macaque
transitional cell	Nokubi (1985)	<i>Suncus</i>

paving-stone appearance, while the microvillous cells (Andrews, 1974), dome-like cell (Kasuya, 1976) and microridge cell (Breipohl *et al.*, 1977) to the cobblestone appearance in this study.

In the *Suncus*, Nokubi (1985) claimed that the intermediate epithelium (pseudostratified nonciliated epithelium in his nomenclature) consisted of the "transitional cells" covered with particle-like microvilli only. Andrews (1974) reported in rat that the microvillous cells had nub-like microvilli on their exposed surfaces. On the other hand, Breipohl *et al.* (1977) described in the rat larynx that there existed the microridge cells characterized by microridges. However, it should be noticed that any types of epithelia may possess both microvilli and microridges, because microridges are formed, or at least contributed to, by a convergence of microvilli (Williams *et al.*, 1973; Saito and Takagi, 1976; Takagi *et al.*, 1976; Takagi, 1977; Breipohl *et al.*, 1977; Hoshika, 1981; Nakano, 1986; Nakano *et al.*, 1986; Nakano and Muto, 1987). Further, Kasuya (1976) distinguished 2 types of the dome-like cells in the human larynx, the small sized-cells with microvilli and the medium-sized cells with microridges, and considered that there was a possibility of the transformation from the small-sized cells to the medium-sized ones. In this study, the intermediate epithelium lining the transitional zone in the *Suncus* larynx was covered with both microvilli and microridges, and various transitional forms between microvilli and microridges could be observed.

It is well known that the larynx is embryo-

logically covered with the ciliated columnar epithelium, and later the epithelium is replaced in part by the stratified squamous epithelium (Hopp, 1955; Uchida, 1961; Endo, 1972). Nakano (1986) suggested that the intermediate epithelium showed the various stages of the epithelium transforming from the ciliated columnar epithelium to the stratified squamous one, and that the transformation represented a protective response to the mechanical stress. According to Podhoranyi *et al.* (1968), locally different pressures produce a physiologic change of the epithelium. In this study, the stratified squamous epithelium was predominantly observed in the epiglottal tip and the region close to the vocal process of the arytenoid cartilage. It is reasonable to assume that these regions are subjected to considerable mechanical stress due to air flow or contact between the epithelial surfaces.

Acknowledgements

The authors would like to express their thanks to Asst. Prof. Dr. J. Kitoh (Nagoya University School of Medicine) for providing the animals.

References

- 1) Ackerman, L.V. and Regato, J.A.: Cancer, diagnosis, treatment, and prognosis. 3rd ed., Mosby, St Louis, 1962.
- 2) Andrews, P.M.: A scanning electron microscopic study of the extrapulmonary respiratory tract.

- Am. J. Anat., **139**: 399-424, 1974.
- 3) Bloom, W. and Fawcett, D.W.: A textbook of histology (Asian ed.), 10th ed., Igaku Shoin, Tokyo, 1976.
  - 4) Breipohl, W., Herberhold, C. and Kerschek, R.: Microridge cells in the larynx of the male white rat. Investigations by reflection scanning electron microscopy. Arch. Oto-Rhino-Laryng., **215**: 1-9, 1977.
  - 5) Copenhaver, W.M.: Bailey's textbook of histology, 5th ed., Williams and Wilkins, Baltimore, 1964.
  - 6) Endo, N.: Histological study on the larynx, its histological change with increasing age (Japanese with English summary). J. Jpn. Broncho-Esophagol. Soc., **23**: 28-50, 1972.
  - 7) Greep, R.O.: Histology, 2nd ed., McGraw Hill, New York, 1966.
  - 8) Ham, A.W.: Histology (Asian ed.), 6th ed., Igaku Shoin, Tokyo, 1969.
  - 9) Hiraki, T.: Scanning electron microscopic observations of the laryngeal mucosa of the Japanese monkey, *Macacus fuscatus* (Japanese with English summary). J. Kurume Med. Ass., **39**: 1-17, 1976.
  - 10) Hopp, E.S.: The development of epithelium of the larynx. Laryngoscope, **65**: 475-499, 1955.
  - 11) Hoshika, K.: The fine structure of esophago-gastric junction (Japanese with English summary). J. Clin. Electron Microsc., **14**: 121-133, 1981.
  - 12) Ichikawa, A., Omochi, S., Shibasaki, S., Setoguchi, T., Nagata, T., Hatai, T. and Yamada, K.: Saishin Soshiki-gaku (in Japanese), 6th ed., Nankodo, Tokyo, 1977.
  - 13) Ishida, M.: Histopathological study on the origins of laryngeal carcinoma (in Japanese). J. Otolaryngol. Jpn., **75**: 550-556, 1972.
  - 14) Kasuya, T.: Electron microscopic investigations of normal and pathological mucosa of the larynx (Japanese with English summary). Otologica Fukuoka, **22**: 299-330, 1976.
  - 15) Kirikae, I.: Modern oto-rhino-laryngology (in Japanese), 6th ed., Nanzando, Tokyo, 1977.
  - 16) Krstić, R.V.: Die Gewebe des Menschen und der Säugetiere. Ein Atlas zum Studium für Mediziner und Biologen (Jpn. ed.), 1st ed., Nishimura, Niigata, 1981.
  - 17) Lewis, D.J.: Mitotic indices of rat laryngeal epithelia. J. Anat., **132**: 419-428, 1981.
  - 18) Lewis, D.J. and Prentice, D.E.: The ultra-structure of rat laryngeal epithelia. J. Anat., **130**: 617-632, 1980.
  - 19) Nakano, T.: Ultrastructural studies of the transitional zone in the nasopharyngeal epithelium, with special reference to the keratinizing process in the mouse. Acta Anat., **127**: 22-47, 1986.
  - 20) Nakano, T. and Muto, H.: Distribution and probable functional role of taste buds located in the intermediate epithelium on the mouse arytenoid region. Okajimas Folia Anat. Jpn., **63**: 81-92, 1986.
  - 21) Nakano, T. and Muto, H.: The transitional zone in the epithelium lining the mouse epiglottis. Acta Anat., **130**: 285-290, 1987.
  - 22) Nakano, T. and Muto, H.: The "intermediate epithelium" lining the mouse larynx. Okajimas Folia Anat. Jpn., **64**: 385-398, 1988.
  - 23) Nakano, T., Muto, H. and Yoshioka, I.: New observations on the fine structure of the normal nasopharyngeal epithelium of the mouse. Auris Nasus Larynx, **13**: 157-168, 1986.
  - 24) Nakano, T., Yoshioka, I. and Muto, H.: Structure and postnatal transformation of the intermediate epithelium lining the mouse nasopalatine duct. Acta Anat., **132**: 132-136, 1988.
  - 25) Nokubi, K.: Anatomy and histology of the house musk shrew. 5. Respiratory organs; in Kondo, K., *Suncus murinus*, Biology of the laboratory shrew (in Japanese), pp. 229-238, Jpn. Sci. Soc. Press, Tokyo, 1985.
  - 26) Nokubi, K. and Igarashi, H.: Laryngeal cartilages in the house musk shrew (*Suncus murinus*). Acta Anat. Nippon, **59**: 187-193, 1984.
  - 27) Nokubi, K., Igarashi, H., Kato, M. and Hayama, S.: Scanning electron microscopic observations on the laryngeal epithelium of macaques. Zool. Mag., **92**: 51-63, 1983.
  - 28) Podhoranyi, G., Valu, L., Sallai, S. and Feher, J.: Über die qualitative Adaptationsfähigkeit des Bindegewebes. A. v. Graefes Arch. Ophthalmol., **175**: 215-222, 1968.
  - 29) Saito, H. and Takagi, T.: Differentiation of the oral lymphoid tissue. Surface structures of the tonsillar epithelium of the developing rabbit (Japanese with English summary). Acta Anat. Nippon, **51**: 168-179, 1976.
  - 30) Sato, T.: Fundamental and clinical studies on the laryngeal diseases (in Japanese). Nihon Iji Shinpo, **2826**: 19-24, 1968.
  - 31) Smith, G.: Structure of the normal rat larynx. Lab. Anim., **11**: 223-228, 1977.
  - 32) Takagi, T.: Surface structure of the normal mucosa. With special reference to the microridge (in Japanese). Dent. J. Iwate Med. Univ., **2**: 125-135, 1977.
  - 33) Takagi, T., Saito, H. and Aso, N.: Mechanism on the differentiation of microridges. Scanning

- electron microscopy of the surface structures of epithelial cells of the developing human tongue (Japanese with English summary). *Jpn. J. Oral Biol.*, **18**: 418-434, 1976.
- 34) Tucker, J.A., Vidic, C., Tucker, G.F. and Stead, J.: Survey of the development of laryngeal epithelium. *Ann. Otol.* 85 Suppl., **30**: 3-16, 1976.
- 35) Uchida, Y.: Histological studies on the larynx. 1. Histological changes in the larynx due to the age and the elapse of time after the death (Japanese with English summary). *J. Tokyo Med. Coll.*, **19**: 2517-2554, 1961.
- 36) Williams, A.E., Jordan, J.A., Allen, J.M. and Murphy, J.F.: The surface ultrastructure of normal and metaplastic cervical epithelia and of carcinoma in situ. *Cancer Res.*, **33**: 504-513, 1973.
- 37) Yamada, K.: Histopathological research on the origins of laryngeal carcinoma (Japanese with English summary). *J. Otolaryngol. Jpn.*, **79**: 175-188, 1976.

Abbreviations used in figures 6-7.

Aa	apex of arytenoid cartilage	LVn	laryngeal ventricle
Ab	base of arytenoid cartilage	LVs	laryngeal vestibule
Av	vocal process of arytenoid cartilage	PR	piriform recess
Ec	epiglottic cartilage	Pt	posterior thyroid cartilage (Nokubi and Igarashi, 1984)
EP	epiglottis	Tb	body of thyroid cartilage
ES	esophagus	Tp	plate of thyroid cartilage
FG	fissure of glottis	VE	vestibular fold
LP	laryngopharynx	VO	vocal fold
LS	laryngeal sac		

Explanation of Figures

Plate I

- Fig. 1. Light micrograph showing a sagittal section of the epiglottis. The rostral surface is lined with the keratinized stratified squamous epithelium (arrowheads), while the caudal surface is covered by the various types of epithelia (arrows). Ep = Epiglottic cartilage. $\times 60$.
- Fig. 2. Micrograph showing the caudal surface of the epiglottis. The stratified squamous epithelium (arrows) continues to the stratified cuboidal type one (arrowheads). $\times 300$.
- Fig. 3. Micrograph showing the caudal surface of the epiglottis. The stratified cuboidal type epithelium (arrowheads) continues to the ciliated stratified low-columnar type one (arrows). $\times 300$.
- Fig. 4. Micrograph showing the laryngeal sac. The ciliated cuboidal or columnar epithelium is seen. $\times 300$.
- Fig. 5. Micrograph showing the vocal fold. The epithelium shows gradations ranging from the ciliated stratified low-columnar type (small arrows) through the stratified cuboidal type (arrowheads) to the stratified squamous type (large arrows). Av = Vocal process of arytenoid cartilage. $\times 200$.

Plate II

Fig. 6. Schematic illustration of a medial section to show the distribution of the epithelium.

Fig. 7. Schematic illustrations of transverse sections to show the distribution of the epithelium. A Section at the base of the epiglottis. B Section at the laryngeal ventricle and the laryngeal sac. C Section at the vocal fold.

Plate III

- Fig. 8. Scanning electron micrograph showing the caudal surface of the epiglottis. In some areas, the surface is covered by the relatively flat and polygonal cells to form the paving-stone appearance (P). In other areas, the superficial cells bulge out from the surface, presenting the cobblestone appearance (C). $\times 1,000$.
- Fig. 9. Micrograph showing the caudal surface of the epiglottis. The ciliated cells (arrows) scatter singly or in small groups among the superficial cells without cilia. $\times 1,000$.
- Fig. 10. Micrograph showing the vestibular fold (VE) and the laryngeal ventricle (LVn). The surface of the vestibular fold shows the paving-stone appearance. $\times 600$.
- Fig. 11. Micrograph showing the laryngeal ventricle (LVn) and the laryngeal sac (LS). As the surface is traced from the ventricle to the sac, the ciliated cells become more numerous. $\times 1,000$.
- Fig. 12. Micrograph showing the vocal fold. The surface covered with cilia (Ci) gradually transforms through the cobblestone appearance (C) to the paving-stone appearance (P). $\times 300$.
- Fig. 13. Higher magnification to show the surface ultrastructure of the intermediate epithelium. A Microvilli. B Microridges. $\times 10,000$.