

정상 Mongolian gerbil의 고막과 외이도상피의 세포증식도

박기현 · 전영명 · 구성모 · 김성균

= Abstract =

Mitotic Activity of Tympanic Membrane and External Auditory Canal Skin in Normal Mongolian Gerbil

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Background : Understanding the differentiation and migrating character of the tympanic membrane, external auditory canal as well as cholesteatoma pathogenesis needs knowledge about epithelial generation center.

Objective : We investigated the distribution of the epithelial generation center in the tympanic membrane and external auditory canal using 5-bromo-2-deoxyuridine(BrdU)immunohistochemistry.

Materials and Methods : BrdU was injected into peritoneum of 10 Mongolian gerbils and gerbil's temporal bones were obtained and serial section was done. Anti-BrdU antibody immunohistochemical stain was done.

Results : The labeling index was highest at posterosuperior quadrant of pars tensa, higher at malleus handle attached area and annulus region. On pars flaccida, labeling index was high at entire area. The labeling index of deep meatal skin was higher than that of lateral meatal skin, especially near annulus area. According to results, epithelial generation centers are thought to be on pars flaccida, posterosuperior quadrant of pars tensa, malleus handle attached area and annulus region.

Conclusion : These findings are similar to those of human tympanic membrane and external auditory canal, and gerbils can be used as a animal model for study of normal human tympanic membrane, external auditory canal and cholesteatomas. (**Korean J Otolaryngol 40 : 7, 1997**)

KEY WORDS : BrdU · Epithelial generation center · Immunohistochemistry · Migration.

서 론

, 1877 Burnett

paper patch

, Litton¹⁾

³H - thymidine uptake

: 1997 3 25

: 1997 6 16

. Alberti²⁾

, Boedts³⁾

재료 및 방법

1. 실험재료

60 75g 90 120
10 Mongolian gerbil(Meriones un-
guiculatus)

2. 실험방법

1) 조직 채취

Gerbil 5 - bromo - 2 - deoxyuridine(Si-
gma Chemical Co. St. Louis, USA) 10mg/kg
ether

50cc 10%

100cc

2) gerbil⁴⁾ 1), Burnett 3), 3), Mongolian gerbil

가

. gerbil

가

10%

gerbil

Mongolian

48 10% EDTA(ethylene dia-
mine tetraacetic acid) 2

가

가

4 μm

ink

, hematoxylineosin

dot method²⁾, ³H - thymidine uptake, BrdU

(Olympus, Venox - S, Japan)

⁵⁾

ink dot me -

thod

BrdU

, BrdU

2) 면역조직화학적 염색

xylene

100, 90, 80, 70%

3%

5

BrdU

. 0.01M, pH 7.6 PBS(phosphate

buffered solution) 5 3 37

0.1% calcium chloride 0.1% trypsin 20

. 0.01M,

pH 7.6 PBS 10%
 10 . 0.01M, pH 7.6 PBS
 95% formamide(Merck Co. Darmstadt, Germany)
 0.15M trisodium citrate 70
 45 double stranded DNA
 . 0.05M, pH 7.6 TBS(trizma buffered so-
 lution) 5 3 20 BrdU 1
 (DAKO Co. Denmark) 12
 . 0.05M, pH 7.6 TBS 2
 10 , 0.05M, pH 7.6 TBS
 ABC(avidin - biotin peroxidase com-
 plex) . 0.05M, pH 7.6 TBS
 AEC(3 - amino - 9 - ethylcarbazole)(DA -

KOCo. Denmark)
 Venox - S, Japan)

(Olympus,

3) 결과 분석

(LI)

Spearman

AEC
 200
 Labeling index
 LI

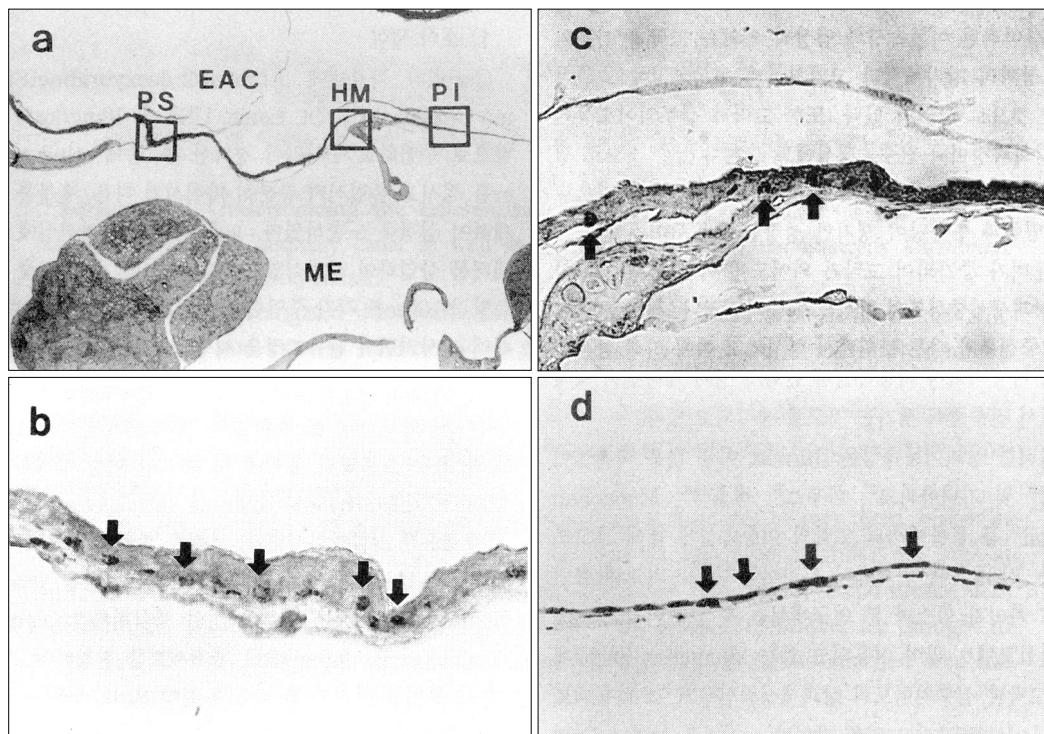


Fig. 1. Light microscopic findings of pars tensa of the tympanic membrane.
 a. Rectangles show posterosuperior quadrant(PS), handle of malleus attached area(HM), postero-inferior quadrant(PI) of the pars tensa of the tympanic membrane(H & E stain, x 40). EAC ; external auditory canal, ME ; middle ear cavity.
 b. BrdU-positive cells(arrows) are shown on the basal cell layer of posterosuperior quadrant of pars tensa (ABC immunostain, x 400).
 c. BrdU-positive cells(arrows) are shown on the basal cell layer of handle of malleus attached area (ABC immunostain, x 400).
 d. BrdU-positive cells(arrows) are shown on the basal cell layer of posteroinferior quadrant of pars tensa (ABC immunostain, x 400).

결 과

10 gerbil 20

BrdU

1. 고 막

(Fig. 1 - a)

(Fig. 1 - b)

가

LI 13.2(4 20)

LI 10.0(4 16)

(Fig. 1 - c) LI 18.0(8 28) (Fig. 1 - d)

LI 3.6(1 10)

가

가

LI 3.2(2 4)

(Fig. 2) BrdU

LI 19.3(8 38)

LI

(Table 1)

가

(p<0.05).

(p>0.05).

2. 외이도상피

(Fig. 3)

LI 12.8(4 20)

LI

11.3(2 20)

가

(Fig. 4)

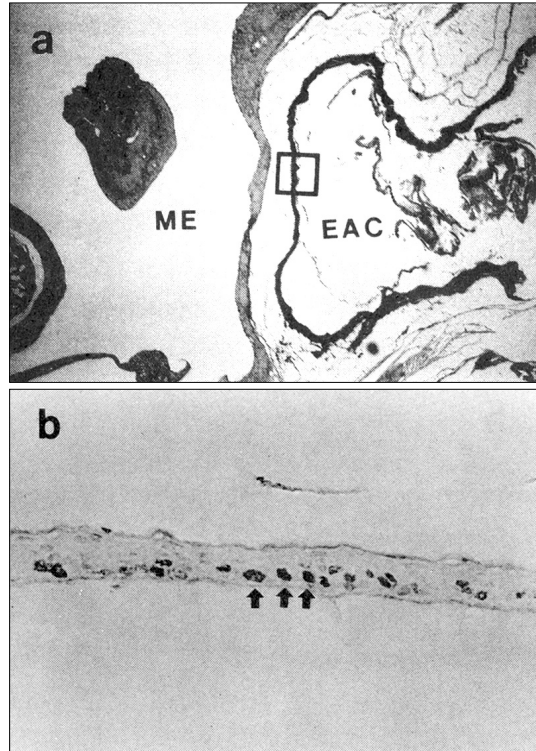


Fig. 2. Light microscopic findings of pars flaccida of the tympanic membrane.
a. Rectangle shows pars flaccida of the tympanic membrane(H & E stain, $\times 40$). EAC ; external auditory canal, ME ; middle ear cavity.
b. BrdU-positive cells(arrows) are shown on the basal cell layer of pars flaccida(ABC immunostain, $\times 400$).

Table 1. Labeling index of normal tympanic membrane in gerbils

Site	LI(mean \pm S.D.)
PSQ	13.2 \pm 5.3
ASQ	10.0 \pm 4.9
PIQ	3.6 \pm 2.6
AIQ	3.2 \pm 1.1
HM	18.0 \pm 7.2
PF	19.3 \pm 12.0

LI : Labeling index(n/200 basal cell)
PSQ : posterosuperior quadrant in pars tensa
ASQ : anterosuperior quadrant in pars tensa
PIQ : posteroinferior quadrant in pars tensa
AIQ : anteroinferior quadrant in pars tensa
HM : handle of malleus attached area
PF : pars flaccida

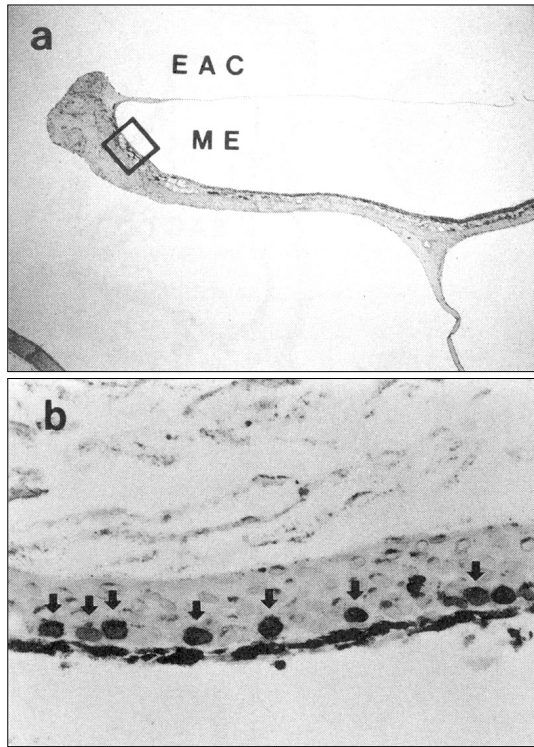


Fig. 3. Light microscopic findings of deep meatal skin. a. Rectangle shows the annulus area of the deep meatal skin(H & E stain, $\times 40$). EAC ; external auditory canal, ME ; middle ear cavity. b. BrdU-positive cells(arrows) are shown on the basal cell layer of the deep meatal skin(ABC immunostain, $\times 400$).

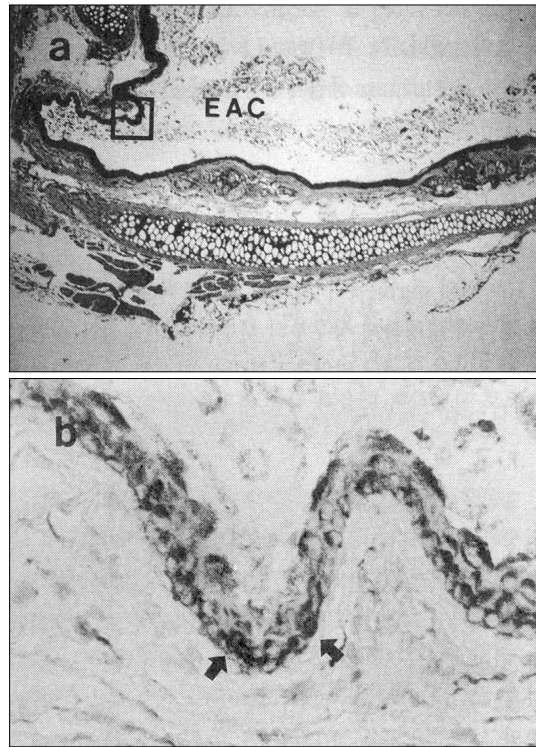


Fig. 4. Light microscopic findings of lateral meatal skin. a. Rectangle shows the lateral meatal skin(H & E stain, $\times 40$). EAC ; external auditory canal. b. BrdU-positive cells(arrows) are shown on the basal cell layer of the lateral meatal skin(ABC immunostain, $\times 400$).

Table 2. Labeling index of normal auditory canal skin in gerbils

Site	LI(mean \pm S.D.)
ADMS	12.8 \pm 4.5
PDMS	11.3 \pm 4.9
LMS	6.3 \pm 2.9

LI : Labeling index(n/200 basal cell)
 ADMS : anterior deep meatal skin
 PDMS : posterior deep meatal skin
 LMS : lateral meatal skin

LI
 6.3(2 11)
 LI (Table 2).

가 ($p < 0.05$).

고 찰

가 6)

가

가 , per patch

1877 Burnett pa-

ink dot method²⁾ ,¹⁾
³⁾, mouse³⁾, Mongolian gerbil⁴⁾ , 가

mouse rat . Alberti²⁾
가 25 70 μm/day
89 157 μm/day . Litton¹⁾

가 , Boedts⁹⁾
stratum corneum .
Johnson¹⁰⁾

filament -
stratum
ous actin
spinosum stratum basale

Yi⁴⁾ 가¹⁾ .
Mongolian gerbil ink
dot method 가

Koba¹¹⁾ 가
BrdU BrdU 가

gerbil Yi⁴⁾ , , ,

ink dot method가
BrdU 가

Gratzner⁸⁾ keratosis¹²⁾ .
BrdU thymidine ,
(S-) BrdU Saad¹³⁾ 가
Sade¹⁴⁾
, Bahadur¹⁵⁾ . Makino¹⁶⁾
가 가

BrdU 가⁷⁾ .
Soucek⁸⁾ 가 Litton¹⁾ 가

가
 gerbil
 18)
 PCNA
 가
 gerbil
 gerbil
 “epithelial generation center”
 가
 가
 Mongolian gerbil
 BrdU gerbil
 BrdU
 propylen glycol chemical irritant ,
 gerbil 가
 Gerbil
 가
 gerbil
 gerbil
 17)
 gerbil

References

- 1) Litton WB : *Epidermal migration in the ear, the location and characteristics for the generation center revealed by utilizing a radioactive desoxyribose nucleic acid precursor. Acta Otolaryngol (Suppl). 1968 ; 240-245*
- 2) Alberti PWRM : *Epithelial migration on the tympanic membrane. J Laryngol. 1964 ; 78 : 808-830*
- 3) Boedts D, Kuijpers W : *Epithelial migration on the tympanic membrane : In experimental study. Acta Otolaryngol (Stockh). 1978 ; 85 : 248-253*

- 4) Yi ZX, Shi GS, Haung CC : *Age-related epithelial migration on the tympanic membrane of the Mongolian gerbil. Otolaryngol Head Neck Surg.* 1988 ; 98 : 564-567
- 5) Gratzner HG : *Monoclonal antibody to 5-bromo- and 5-iododeoxyuridine. Science.* 1982 ; 218 : 474-475
- 6) Johnson AP, Hawke M, Berger G : *Surface wrinkles, cell ridges and desquamation in the external auditory canal. J Otolaryngol.* 1984 ; 13 : 345-354
- 7) Lee HM, Kim MS, Choi G, Hwang SJ : *Immunohistochemical study on the turnover of the tongue epithelium using anti-BrdU antibody in normal mice. Korean J Otolaryngol.* 1992 ; 35 (1) : 80-87
- 8) Soucek S, Michaels L : *Auditory epithelial migration : The existence of two discrete pathways and their embryologic correlates. Am J Anat.* 1990 ; 189 : 189-200
- 9) Boedts D, Ridder L, Cock M : *Tympanic keratin dispersion and epithelial spreading. In : Cholesteatoma and mastoid surgery, 3rd International Conference. Amsterdam : Kugler and Ghedini Publications, 1988 : 278-283*
- 10) Johnson AP, Boden P, Weinberger JM : *In situ localization of F-actin in the normal and injured guinea pig tympanic membrane. Acta Otolaryngol (Stockh).* 1986 ; 101 : 278-285
- 11) Koba R : *Epidermal cell migration and healing of the tympanic membrane : An immunohistochemical study of cell proliferation using bromodeoxyuridine labeling. Ann Otol Rhinol Laryngol.* 1995 ; 104 : 218-225
- 12) Freedberg IM : *Epidermal differentiation and keratinization. In : Cholesteatoma, 1st International Conference, McCabe et al. Birmingham : Aesculapius Publications, 1977 : 38-44*
- 13) Saad EF : *The epidermis of the drumhead in some otologic conditions. Arch Otolaryngol.* 1977 ; 103 : 387-388
- 14) Sade J : *Retraction pockets and attic cholesteatomas. Acta Otorhinolaryngol (Belg).* 1980 ; 34 : 62-84
- 15) Bahadur S, Kacker SK : *Epithelial migration in the posterosuperior retraction pocket and in grafted tympanic membranes. Ear Nose Throat.* 1982 ; 61 : 98-101
- 16) Makino K, Amatsu M : *Epithelial migration in retraction pocket and cholesteatoma. In : Cholesteatoma and mastoid surgery, 3rd International Conference. Amsterdam : Kugler and Ghedini Publications, 1988 : 313-316*
- 17) McGinn MD, Chole RA, Henry KR : *Cholesteatoma : experimental induction in the mongolian gerbil, meriones unguiculatus. Acta Otolaryngol (stockh).* 1982 ; 93 : 61-67
- 18) Chun YM, Park KH : *Hyperproliferative characteristics in human deep meatal epidermis. Korean J Otolaryngol.* 1997 ; 40 (1) : 56-62