

-defensin

-defensin

2000

12

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|                    |          |         |
|--------------------|----------|---------|
|                    | .....    | 1       |
| I.                 | .....    | 2       |
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|    |          |             |                       |
|----|----------|-------------|-----------------------|
| 1. |          | RT-PCR      |                       |
|    | hBD-1    | 2 mRNA      | ..... 6               |
| 2. | human    | -defensin 1 | 2 mRNA RT-PCR ..... 6 |
| 3. | hBD-1    | mRNA        | RT-PCR ..... 7        |
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**-defensin**

가 , 가  
 가 , 가  
 가 human -defensin (hBD)  
 hBD 가  
 , hBD  
 가 가  
 , hBD-1 specific primer hBD-2 specific primer total RNA  
 . PCR house keeping gene RT-PCR  
 densitometer  
 IL-8 mRNA  
 hBD mRNA  
 (HEI-HMEEC cell line) TNF- IL-1  
 hBD-1 mRNA hBD-2 mRNA  
 hBD-1 mRNA , hBD2 mRNA  
 hBD-1 hBD-2 mRNA 가 ,  
 가 가  
 IL-1 TNF- hBD-1 mRNA  
 , hBD-2 mRNA 가  
 , hBD 가  
 , hBD-1  
 , hBD-2  
 가

---

: human beta-defensin, RT-PCR, , ,





“ ”

가

(host innate immunity)

5-7)

(normal flora)

가

defensin family<sup>8-11)</sup> defensin family cystein disulfide

-defensin -defensin

Paneth cell

-defensin

defensin

TAP (tracheal antimicrobial peptide)가

LAP (Lingual antimicrobial peptide) 가

, human -defensin-1 2가 hBD-1 2

cystic fibrosis 가 cystic

fibrosis 가

12)

-defensin 가 hBD 가

, hBD가

## II.

### 1.

가 가 , -80°C RNA ,

### 2.

, human papilloma virus oncogene  
E6/E7 hybrid gene transfection (HEI-  
HMEEC cell line) <sup>13)</sup> 70 ,  
20 30  
Bronchial/Tracheal Epithelial Cell Growth  
Medium (BEGM) (Clonetics, Walkersville, MD, USA) BEGM  
가 13 mg/ml BPE (Bovine Pituitary Extract), 2 ml; 0.5 mg/ml Hydrocortisone,  
0.5 ml; 0.5 mg/ml hEGF (human recombinant Epidermal Growth Factor), 0.5 ml; 0.5 mg/ml  
Epinephrine, 0.5 ml; 10 mg/ml Transferrin, 0.5 ml; 5 mg/ml Insulin, 0.5 ml; 0.1 mg/ml Retinoic  
Acid, 0.5 ml; 6.5 mg/ml Triiodothyronine, 0.5 ml; 50 mg/ml Gentamicin, 50 mg/ml  
Amphotericin-B 2 , 37°C 5%  
CO<sub>2</sub>

### 3. TNF- IL-1

2  $6 \times 10^4$  6 well plate , 75 90% (confluence)  
12  
(BEGM)  
. TNF- (Sigma, Saint Louis, MI, USA) IL-1 (Sigma, Saint Louis, MI, USA) 1% BSA  
10, 50 ng/ml 6  
0.25% trypsin/0.01% EDTA (Bio-Whittaker, San Diego, CA, USA)

TNF- IL-1 1% BSA

#### 4. RNA preparation

liquid nitrogen  
, lysis buffer homogenizer , Rneasy mini kit (Qiagen, Santa Clarita, CA.  
U.S.A) , total RNA Kit  
total RNA

#### 5. RT-PCR

Random primer Superscript II RT (Life technologies, Grand Island, NY, U.S.A)  
1 g total RNA 1<sup>st</sup> stranded cDNA . cDNA primers  
(PCR) . hBD-1 specific primer 5': TTG-  
TCTGAGATGGCCTCAGGTGGTAAC, 3': TTTACTTCTGCGTCATTTCTTCTGG , hBD-2  
specific primer 5'-TTTGGTGGTATAGGCGATCC, 3'-ATGTCGCACGTCTCTGATGA .  
house keeping gene beta 2-microglobulin ( 2M) primer 5'-CTCGCGCT-  
ACTCTCTCTTTCTGG, 3'-GCTTACATGTCTCGATCCCACTTAA , IL-8 pri-  
mer 5'-ATGACTTCCAAGATGGCCGTGGCT, 3'-TCTCAGCCCTCTTCAAACCTTCTC  
DNA thermal cycler (MJ Reaserch, INC, Waltham,  
MA, USA) 94°C 1 (denaturation) , primer  
(annealing) , 72°C (polymerization) 3 1  
25 35 primer  
primer 25 35 2 가 가  
primer (TM)  
, hBD-1 58°C 200 bp, hBD-2 60°C 200 bp , IL-8  
55°C 298 bp, 2M 58°C 335 bp 0.1  
mg/ml ethidium bromide가 1.2% agarose gel , PCR  
product ABI PRISM genetic analyzer (Applied Biosystems, Foster City, CA, U.S.A)

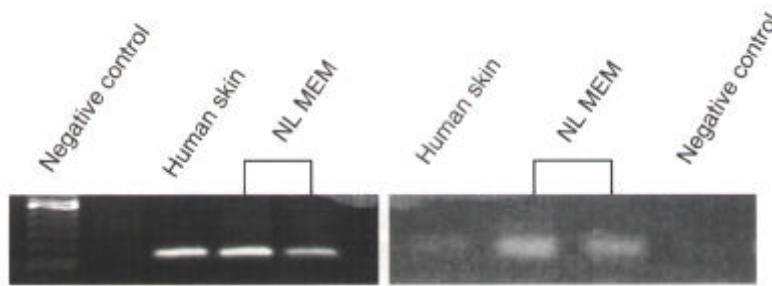
#### 6. PCR

Beta-microglobulin gene mRNA densitometer (Molecular  
dynamics, Sunnyvale, CA, U.S.A)  
IL-8 mRNA hBD mRNA ,

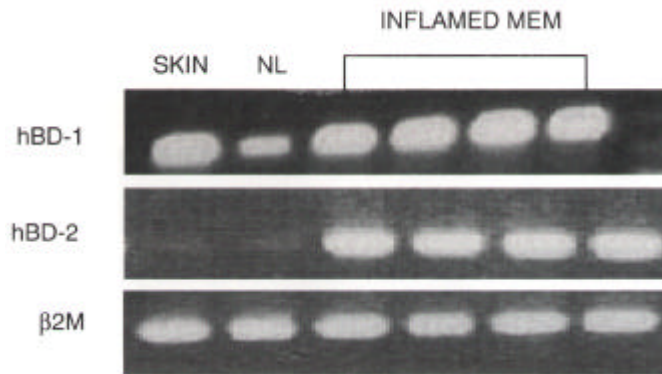
III.

1. hBD mRNA

hBD-1 hBD-2 gene mRNA ( constitutive gene



1. RT-PCR hBD-1 2 mRNA .  
 constitutive gene  
 hBD-1 gene hBD-2 gene mRNA  
 (NL MEM: normal middle ear mucosa).



2. human  
 -defensin 1 2 mRNA RT-PCR .  
 -defensin 1  
 10 4 가 가  
 hBD-2 mRNA  
 가 . NL: normal, MEM: middle ear mucosa, 2M: beta  
 2 microglobulin.

hBD-1

HEI-HMEEC

hBD-2 gene mRNA

hBD-1 mRNA hBD-2 mRNA

HBD-2 mRNA

hBD-1 mRNA

mRNA

hBD-2

가

2.

hBD mRNA

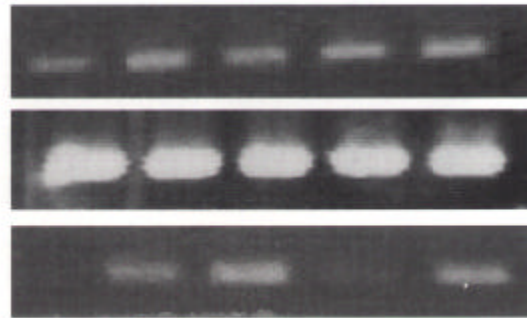
hBD

NL #1 #2 #3 #4

hBD-1

$\beta$ 2M

IL-8



3.

hBD-1 mRNA

RT-PCR

hBD-1

가

가

IL-8 mRNA

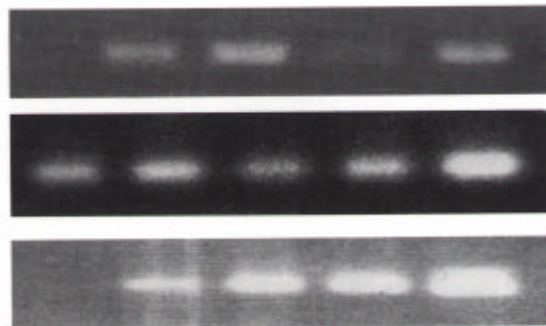
NL: normal, #: specimen number.

NL #1 #2 #3 #4

h  $\beta$ D-2

$\beta$ 2M

IL-8



4.

hBD-2 mRNA

RT-PCR

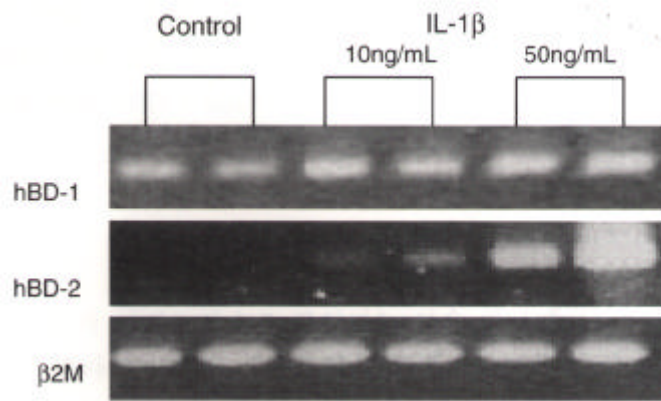
hBD-2

가

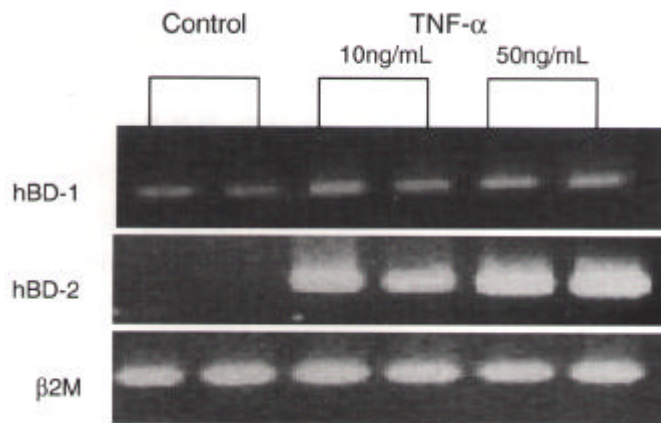
IL-8 mRNA

NL: normal, #: specimen number.

hBD-1 hBD-2 mRNA  
 hBD-1 10 4 가  
 가 hBD-2 mRNA 가  
 ( 2). IL-8 mRNA hBD-1 mRNA  
 hBD-2 mRNA IL-8 mRNA 가 ( 3, 4).



5. IL-1 HEI-HMEEC hBD-1, hBD-2 가 .  
 2 mRNA . IL-1 HEI-HMEEC hBD-1  
 10, 50 ng/ml 6  
 hBD-2 mRNA 가 .



6. TNF-α HEI-HMEEC hBD-1, hBD-2 가 .  
 2 mRNA . 10, 50 ng/ml TNF-α hBD-1  
 hBD-2 mRNA 가 . TNF-α hBD-1

3. hBD mRNA

HEI-HMEEC TNF- IL-1 hBD mRNA  
 mRNA . TNF- hBD-1 mRNA hBD  
 , hBD-2 mRNA 가 . 10, 50 ng/ml 6  
 hBD-2 mRNA 가 ( 5). IL-1 TNF- . 10, 50 ng/ml  
 hBD-2 mRNA 가 ,  
 가 . hBD-1 mRNA  
 ( 6).

IV.

Defensin 100 가  
 . defensin  
 ,  
 ,  
 13,14) . defensin  
 ,  
 ,  
 defensin  
 . -defensin defensin  
 ,  
 (dendrite cell) T  
 가 .  
 ,  
 -defensin  
 14) . defensin  
 .  
 hBD-1 , ,  
 hBD-2 mRNA 가  
 15) . -defensin 1 2 mRNA가  
 ,  
 -defensin

mRNA

, 가

lactoferrin, surfactant protein,

sIgA

, lysozyme,

2).

가

-defensin

가 defensin

, defensin

가

-defensin

가

defensin

hBD

. hBD-1

constitutive defensin

. hBD-2

inductive defensin

15-18)

mRNA가

, hBD-2

, hBD-1

mRNA가

hBD-1

mRNA hBD-2 mRNA

가

, hBD-2 mRNA

가

, hBD-1 mRNA

가

. in

*vitro*

, IL-1

TNF-

hBD-2 mRNA

가

, hBD-1 mRNA

가

defensin

defensin

hBD-1 mRNA

가

가

Krisannaprakornkit

hBD-1

nuclear factor kB

가

, bovine -defensin

가

19).

E. coli LPS TNF-



, hBD-1 가 , hBD-1 가  
tracheal-derived antimicrobial peptides (TAP)  
가 human -defensin gene nuclear factor-IL-6 gamma  
interferon consensus site hBD-1

hBD-1 mRNA가 가  
가 가 가  
HBD-2 inductive defensin , , ,  
RNA (15,20,21)  
hBD-2 mRNA  
RT-PCR  
hBD-2  
(7,12,22,23)  
가 Harder hBD-2가  
가 (15)  
hBD-1 hBD-2가 LAP TAP  
가 hBD-1 2  
hBD-2  
mRNA 가 IL-8  
mRNA , HEI-HMEEC  
Interleukin-1 TNF- 가  
*in vitro in vivo*  
defensin 가  
가 가  
hBD Bøe  
hBD-1 mRNA , RT-PCR  
hBD-1 mRNA가 (24)  
*in situ* hybridization  
hBD-1  
hBD-1

hBD-1 mRNA가

hBD-1

가

hBD-1

hBD-2가

가

가

가

V.

(HEI-

HMEEC cell line)

hBD

1.

hBD-1 mRNA

, hBD-2 mRNA

2.

hBD-2 mRNA

가 , hBD-1

가

가

3.

IL-1 TNF-  
, hBD-2 mRNA

hBD-1 mRNA  
가

hBD

hBD-1

, hBD-2

mRNA

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## Abstract

### **Human $\alpha$ -defensin genes expression in normal and inflamed middle ear epithelium**

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(Directed by Professor Myung-Hyun Chung)

I believe that several secretory products such as lysozyme, lactoferrin, and other unknowns might play an important role in protection against pathogen and maintenance of the immunity in tubotympanum. Human  $\alpha$ -defensin (hBDs) is antimicrobial peptides that may play a role in mucosal defense. To define the role of defensin in middle ear cleft, I analyzed the expression and regulation of two human  $\alpha$ -defensin genes (hBD-1, hBD-2) in human middle ear epithelial tissue and human middle ear epithelial cell line (HEI-HMEEC cell line) by using RT-PCR. The normal human middle ear epithelium expressed hBD-1 mRNA but a little hBD-2 mRNA. While hBD-2 mRNA was markedly up-regulated in inflamed middle ear epithelium, the expressions of hBD-1 mRNA were slightly elevated in four cases of ten inflamed middle ear mucosa, other of which were similar with those of control mucosa. On the other hand, the pro-inflammatory cytokines such as TNF- $\alpha$  and IL-1 $\beta$  stimulated the expression of hBD-2 mRNA, but not hBD-1 mRNA in HEI-HMEEC cell line.

The present study might be a first report to identify human  $\alpha$ -defensin genes expression at mucosal surface in human middle ear tissue and cell line. Moreover, these genes may play an important role in maintenance of middle ear innate immunity. These data suggest that in middle ear hBD-2 expression is induced by inflammation, whereas hBD-1 may serve as a defense in the absence of inflammation. Furthermore, these results provide insights into defining mechanisms of dysfunction of tubotympanum and allow better idea of its treatment and prevention of otitis media.

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Key Words: human  $\alpha$ -defensin gene, otitis media, innate immunity, middle ear epithelium, RT-PCR