

고농도 EGF로 처리된 KUMA3 세포주의 세포유전학적 연구

채한수¹ · 송달원¹ · 정현수¹ · 김희준¹ · 홍창의¹ · 안병훈¹ · 최인장²

Cytogenetic Studies of High Dose EGF Treated KUMA3 Cell Clone

Han Soo Chae, MD¹, Dal Won Song, MD¹, Hyun Soo Jeong, MD¹, Hee Jun Kim, MD¹,
Chang Eui Hong, MD¹, Byung Hoon Ahn, MD¹ and In Jang Choi, PhD²

¹Department of Otolaryngology, ²Anatomy, College of Medicine, Keimyung University, Taegu, Korea

ABSTRACT

Background and Objectives : Epidermal growth factor (EGF), directly stimulates epidermal growth and differentiation. The combination of EGFR activation and genetic alternations may lead to neoplasia and metaplasia. To study the change of chromosomal number and the aberrations of chromosomal structure of KUMA3 cell line treated with high dose EGF. **Materials and Methods :** The high dose EGF treated cell clones were obtained from KUMA3 cell line which was established from squamous cell carcinoma of the lower lip by culturing cells in medium containing high dose EGF for 6 months. The chromosomal analysis and subculture were performed at subsequent passage of 1 month interval. **Results :** In high dose EGF treated cell clones, there was no apparent change in chromosomal number, but the ratio of the number of chromosome 7 to mode chromosome number was similar to normal value (0.043). The new chromosomal structural aberrations appeared first from 30 passage of IR-200 cell clone. The chromosomal aberrations were del(1)(q23 qter) and del(4)t(1 : 4)(1qter 1q23 : 4p16 4qter). **Conclusion :** There was no change in chromosomal number, but the ratio of the number of chromosome 7 to mode chromosome number was similar to normal value (0.043), and the new chromosomal structural aberrations were appeared. (Korean J Otolaryngol 1998;41 (2):232-237)

KEY WORDS : EGF · KUMA3 cell clone · Squamous cell carcinoma · Chromosomal aberrations.

EGF(epidermal growth factor) 53
 polypeptide
 Carpenter Cohen¹⁾ A431 Gill⁵⁾ EGF A431
²⁾ ³⁾ 7 EGF
 EGF가 A431 가 7
 EGF 가 가
 EGF EGF
 가 EGF Gill Lazor²⁾ tyrosine
 가
 : 1997 4 14 / : 1997 10 20 EGF⁶⁾ EGF⁷⁾
 : , 700 - 712 194 가
 : (053) 250 - 7715 · : (053) 256 - 0325

Khazaie ⁸⁾ autocrine paracrine EGF 200) EGF KUMA3

31 ml 600 ng EGF 가 6 36 가

EGF 가 KUMA3 EGF 세포유전학적 분석 KUMA3 EGF

가 7 가 EGF paracrine colcemide(Gibco , USA) 37

가 (200 ng/ml 600 ng/ml) EGF 가 EGF 60 6

EGF 6 EGF 0.025% (Difco , USA) 5 10

KUMA3 가 7 EGF 가 10% H₂O₂ (Sigma , USA) 4% Giemsa (Merck , USA) G - banding

5 10 50 20 ISCN⁹⁾

24 36

KUMA3 KUMA3 EGF 7

EGF EGF 가

KUMA3 세포주 배양과 고농도 EGF 처리 변이주 확립 KUMA3 10% (Gibco , USA) EGF 가 36

F10(Gibco , USA) 3 (Fig. 1 and Table 1) 7 가 0.043

24 KUMA3 ml (Table 1). EGF

200 ng EGF(Sigma , USA) 가 (1R - 1R - 200 26 7 가

200) 3 5 EGF가 2.36 7

1 29 0.036

고농도 EGF 처리 변이주의 특이한 구조적 염색체이상 출현 이후의 배양 7 (Table 1). EGF

EGF (1R - 200) 30 1 1

ml 200 ng EGF가 가 (2R - 200), 4

ml 600 ng EGF가 가 (2R - 600) (Fig. 2) EGF (1R - 200)

EGF 가 (2R - 0) 30 2R - 200, 2R -

EGF (1R - 200) 600 2R - 0 7 가

ml 200 ng EGF 가 (1R -

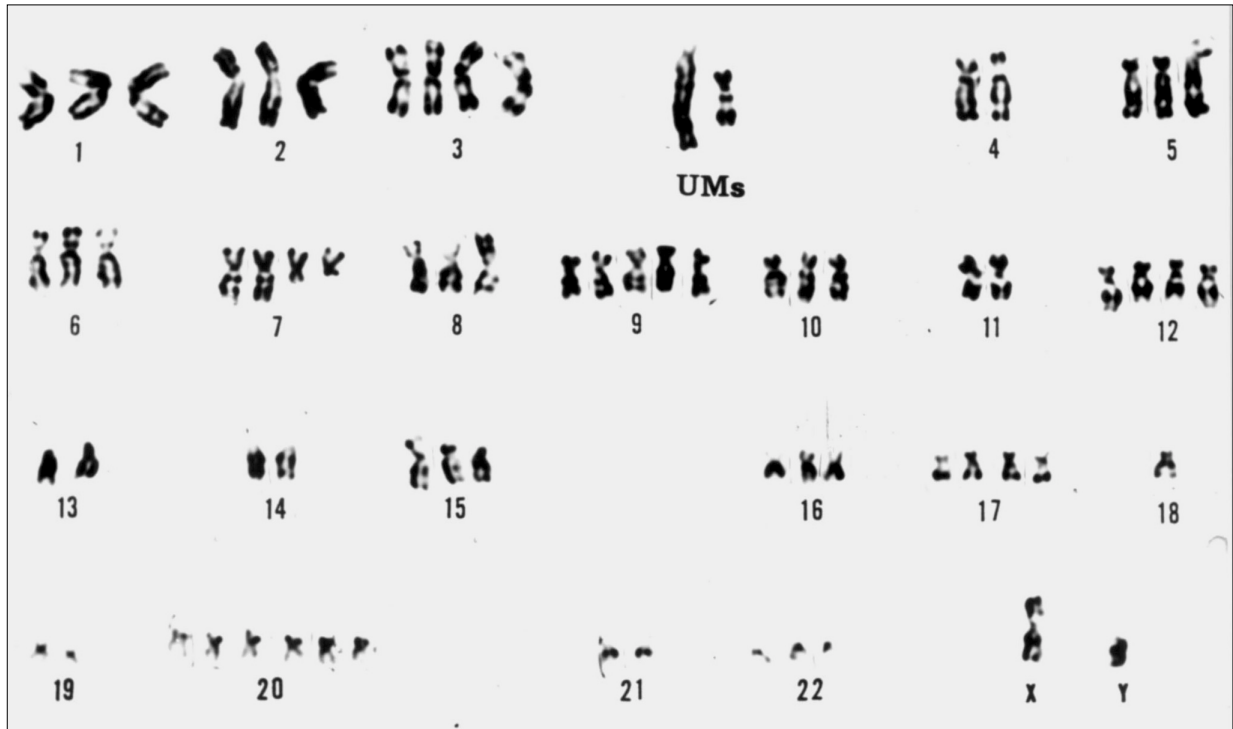


Fig. 1. Representative karyotype of the EGF sensitive parental cell line KUMA3 at passage 26. UMs, unidentified marker chromosome.

Table 1. Comparison of the mode chromosome number (A), the number of chromosome 7 (B) and the ratio of the number of chromosome 7 to mode chromosome number (B/A) at each passage in KUMA3 cell and its variants

Passage No.	26			29			32			36		
Clone	A	B	B/A	A	B	B/A	A	B	B/A	A	B	B/A
KUMA3	69.95	3.25	0.046	66.65	3.30	0.050	64.43	3.00	0.046	66.55	4.40	0.066
1R-200	64.64	2.36	0.036	65.83	2.83	0.042	-	-	-	-	-	-
2R-200	-	-	-	-	-	-	61.74	2.57	0.041	55.00	2.62	0.045
2R-600	-	-	-	-	-	-	62.81	2.57	0.042	59.86	2.71	0.045
2R-0	-	-	-	-	-	-	65.00	2.91	0.044	65.74	2.84	0.043
1R-600	-	-	-	-	-	-	-	-	-	65.40	2.78	0.043

(Table 1).

KUMA3 30 ml 600 ng
EGF 가 EGF
가 36 1R-600 (Fig. 4)
7 가
(Table 1).

EGF
24 KUMA3 EGF
6 30 1R-
200 KUMA3
del(1)(q23 qter) der(4)
t(1:4)(1qter 1q23::4p16 4qter)(Fig. 2)가

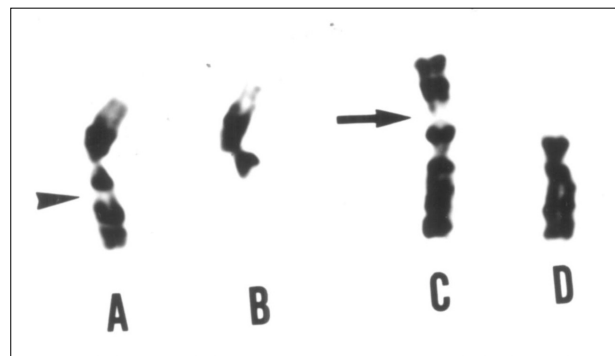


Fig. 2. Partial karyotype illustrating the new structural rearrangements of the high dose EGF treated cell clone 1R-200 at passage 30. A : a normal chromosome 1 ; B : del(1)(q23 qter) ; C : der(4)t(1:4)(1qter 1q23::4p16 4qter) ; D : a normal chromosome 4. The arrowhead and arrow indicate breakpoint and translocation, respectively.

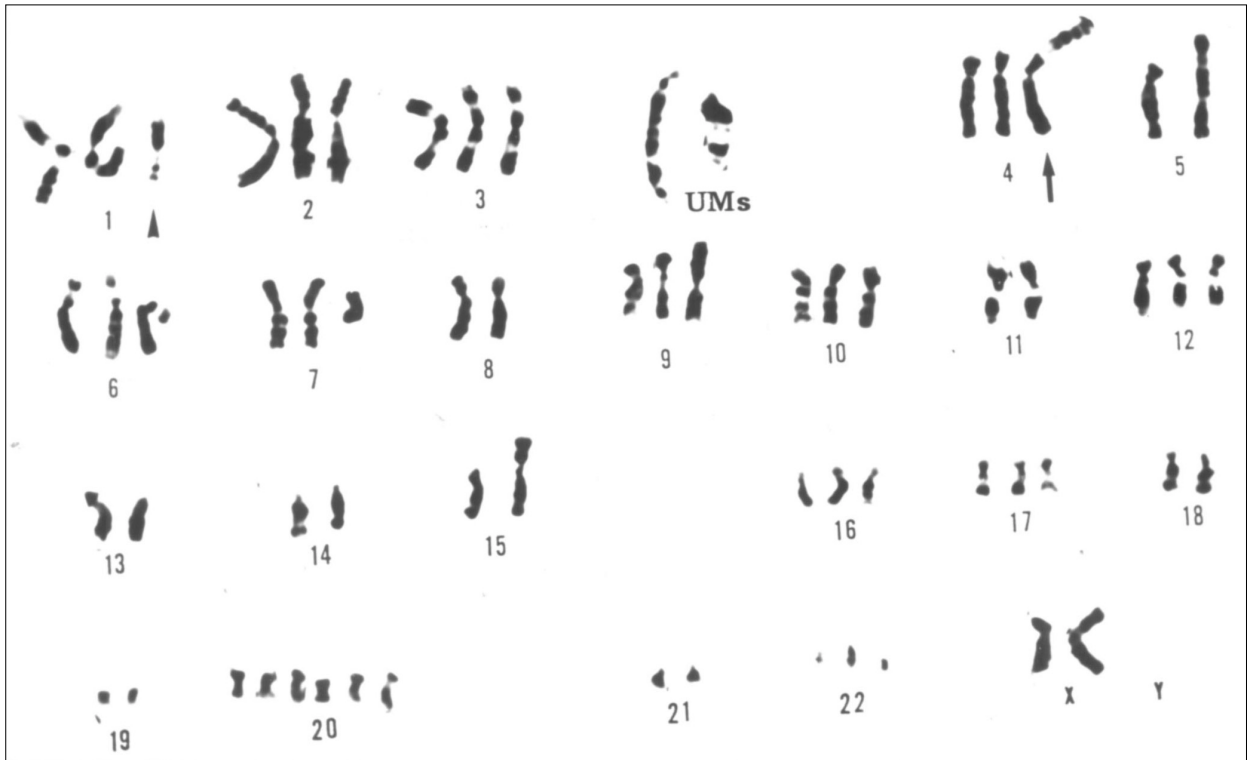


Fig. 3. Representative karyotype of the high dose EGF treated cell clone 2R-200 at passage 32. The arrowhead and arrow indicate del(1)(q23-qter) and der(4)t(1:4)(1qter-1q23::4p16-4qter), respectively. UMs, unidentified marker chromosome.

Table 2. Percentage of cells maintaining del(1)(q23-qter) [D], der(4)t(1:4)(1qter-1q23::4p16-4qter) [T], both [B] and none [N] at each passage in KUMA3 and high dose EGF treated cell clones

Passage No.	32				36			
	D	T	B	N	D	T	B	N
KUMA3	0	0	0	100	0	0	0	100
2R-200	5	20	75	0	5	10	85	0
2R-600	5	5	85	5	10	5	85	0
2R-0	0	0	10	90	5	0	25	70
1R-600	-	-	-	-	50	0	0	50

2R - 200(Fig. 3) 2R - 600 del(1)(q23-qter) der(4)t(1:4)(1qter-1q23::4p16-4qter) 가 100% (Table 2).
 2R - 0 32 가 10% 36 del(1)(q23-qter) 가 30% (Table 2).
 가 36 1R - 600 30 1R - 200 del(1)(q23-qter) 7

(Fig. 4)

50%

(Table 2).

EGF

EGF

EGF

A431

2)

MDA - 468

10)

JSQ - 3

11)

EGF

EGF

EGF

EGF

EGF

EGF

EGF

EGF

tyrosine

kinase

가

tyrosine

kinase

EGF

가

4)

Gill

5) EGF

A431

EGF

가

7

36

1R - 600

30

1R - 200

M4

M14

가

7

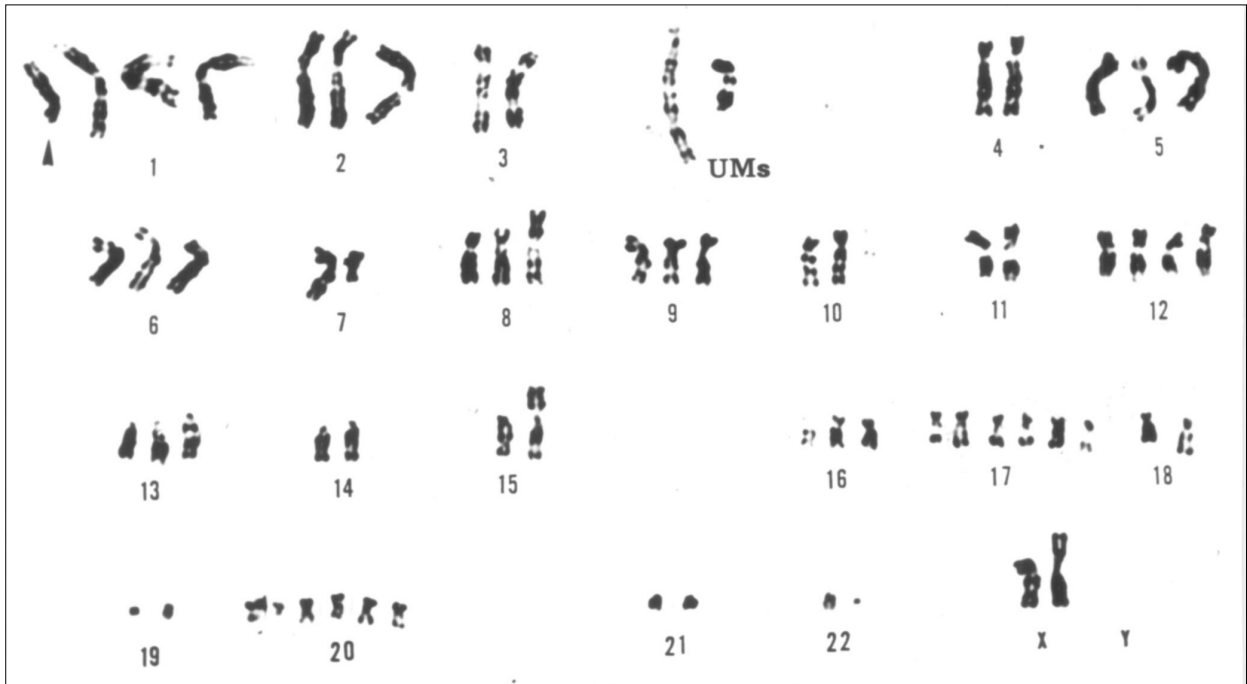


Fig. 4. Representative karyotype of the high dose EGF treated cell clone 1R-600 at passage 36. The arrowhead indicates del(1)(q23-qter). UMs, unidentified marker chromosome.

Filmus¹²⁾ EGF 가
 MDA - 468 EGF 가 ,⁶⁾⁷⁾ Khazaie⁸⁾ autocrine paracrine
 7 (abnormally banded EGF가 EGF
 region)가 가 ,
 EGF
 KUMA3 EGF KUMA3 EGF
 , EGF 가 7 가 del(1)(q23-qter) der
 7 가 가 EGF 30 1q23 : : 4p16 4qter
 EGF 2R - 600
 7 가 EGF 가
 26 IR - 200 0.036 100% EGF 가 2R -
 EGF tyrosine 0 가
 kinase 가 7 가 가 1R - 600 6
 가 7 가 가 del(1)(q23-qter)
 0.043 Filmus¹⁰⁾ 50%
 EGF EGF 가 EGF 6 가
 EGF가 del(1)(q23-qter) EGF
 tyrosine del(1)(q23-qter)
 EGF EGF 1 22 24

Chaganti 가 14) 1 22 24 ski
 가 ski
 EGF Santini 15)
 EGF 가
 EGF 가 EGF
 가 7 가
 0.043 del(1)(q23 qter) 가
 KUMA3
 EGF KUMA3
 EGF 6
 KUMA3 EGF
 가 EGF
 KUMA3 30 del
 1R - 200 (1)(q23 qter) der(4)t(1 : 4)(1qter 1q23 : :
 4p16 4qter)
 2R
 30 EGF 1R - 600
 del(1)(q23 qter)
 : KUMA3

REFERENCES

- 1) Carpenter G, Cohen S, Gill GN. *Epidermal growth factor. Annu Rev Biochem* 1979;48:193-216.
- 2) Gill GN, Lazar CS. *Increased phosphotyrosine content and inhibition of proliferation in EGF-treated A431 cells. Nature* 1981; 293:305-7.
- 3) Kamata N, Cahida K, Rikimaru K, Horikosch M, Enomoto S, Kuruki T. *Growth inhibitory effects of epidermal growth factor and overexpression of its receptors and human squamous cell carcinoma in culture. Cancer Res* 1986;46:1648-53.
- 4) Buss JE, Kudlow JE, Lazar CS, Gill GN. *Altered epidermal growth factor (EGF)-stimulated protein kinase activity in variant A431 cells with altered growth responses to EGF. Proc Natl Acad Sci USA* 1982;79:2574-8.
- 5) Gill GN, Weber W, Thompson DM, Lin C, Evans RM, Rosenfeld MG. *Relationship between production of epidermal growth factor receptors, gene amplification, and chromosome 7 translocation in variant A431 cells. Somatic Cell and Molecular Genetics* 1985; 11:309-18.
- 6) Stern DF, Hare DL, Cecchini MA, Weinberg RA. *Construction of a novel oncogene based on synthetic sequences encoding epidermal growth factor. Science* 1987;235:321-4.
- 7) Heidaran MA, Fleming TP, Bottaro DP, Bell GI, Fiore PPD, Aronson SA. *Transformation of NIH3T3 fibroblasts by an expression vector for the human epidermal growth factor precursor. Oncogene* 1990;5:1265-70.
- 8) Khazaie K, Schirmmcher V, Lichtner RB. *EGF receptor in neoplasia and metastasis. Cancer and Metastasis Reviews* 1993;12: 255-74.
- 9) ISCN. *An international system for human cytogenetic nomenclature. Cytogenet Cell Genet* 1987;21:309-404.
- 10) Filmus J, Pollak MN, Cailleau R, Buick RNI. *MDA-468, a human breast cancer cell line with a high number of epidermal growth factor (EGF) receptors, has an amplified EGF receptor gene and is growth inhibited by EGF. Biochem Biophys Res Commun* 1985; 128:898-905.
- 11) Weichselbaum RR, Dunphy EJ, Beckett MA, Tyber AG, Moran WJ, Goldman ME, et al. *Epidermal growth factor gene amplification and expression in head and neck cancer cell lines. Head and Neck* 1989;11:437-42.
- 12) Filmus J, Trent JM, Pollak MN, Buick RN. *Epidermal growth factor receptor gene-amplified MDA-468 breast cancer cell line and its nonamplified variants. Mol Cell Biol* 1987;7:251-7.
- 13) Koduru PRK, Filippa DA, Richardson ME, Jhanwar SC, Chaganti SR. *Cytogenetic and histologic correlations in malignant lymphoma. Blood* 1987;69:97-102.
- 14) Chaganti RS, Balazs I, Jhanwar SC, Hurfy VV, Koduru PR, Grzeschik KH, et al. *The cellular homologue of the transforming gene of SKV avian retrovirus maps to human chromosome region 1q22-24. Cytogenet Cell Genet* 1986;43:181-6.
- 15) Santini J, Formento JL, Francoual M, Milano G, Schneider M, Dassonville O, Demard F. *Characterization, quantification and potential clinical value of the epidermal growth factor receptor in head and neck squamous cell carcinomas. Head and Neck* 1991; 13:132-9.