Detecting the (Epidermal Growth Factor Receptor) EGFR Gene Amplification in Oral Carcinogenesis

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Abstract: Abnormal amplification of the epidermal growth factor receptor (EGFR) gene has been reported in various human tumors. We used competitive polymerase chain reaction (PCR) to study weather EGFR gene is amplified and the degree of amplification .We used in this study 17 cases of oral epithelial dysplasia (ED), 4 cases of carcinoma in Situ (CIS) and 20 cases of primary squamous cell carcinoma SCC. The extracted DNA was subjected to competitive PCR to amplify EGFR gene . Amplification of EGFR gene was observed in 3 cases (17%) of ED, 1 case of CIS and 4 cases (20%) of SCC. The degree of amplification was low in ED and CIS but extremely high in SCC. This suggests that amplification of EGFR occurs in early stage of oral SCC however high levels of EGFR plays a role in the progression to invasive carcinoma.

Key words: Epidermal growth factor receptor, Squamous cell cancer, gene amplification, polymerase chain reaction.

Introduction

Epidermal growth factor receptor (EGFR) is a widely studied tyrosine kinase receptor, which plays a role in the control of cell proliferation. Abnormalities of EGFR gene and over expression of the protein have been reported in various human tumors. Abnormal amplification of EGFR gene is a relative frequent in SCC of the head and neck, esophagus, and lung cancer. Differential polymerase chain reaction (PCR) permits studies of gene amplification using minute quantities of genomic DNA. Contrary to differential PCR, competitive PCR permits not only determination of absence or presence of amplification, but also the degree of amplification. In this study we used competitive PCR in to investigate the amplification and the degree of amplification of EGFR gene in oral epithelial dysplasia ED, carcinoma in situ (CIS) and early stage SCC, in an attempt to study the correlation between EGFR amplification and development of SCC.

Materials and methods

Formaline fixed specimens of 18 cases of oral ED, 5 cases of CIS and 20 cases of early stage SCC and six cases of normal epithelium as controls. The sequence of the primers used and the sizes of PCR products obtained are shown in (Table.1). A competitive DNA fragment was constructed by restriction enzymes from the 110 bp amplified region of EGFR resulting in an 87 bp fragment.Genomic DNA extracted from normal human leukocytes was used as the standard sample. The quantity of competitive DNA to be used in the gene amplification study was adjusted to be equal to the number of copies of EGFR gene in 40 ng of the reference sample based on OD value, by the following procedure .Serially diluted competitive DNA and 40 ng of genomic DNA of the standard sample were added simultaneously to the PCR reaction mixture, and were amplified while competing for the same EGFR primer (Table.2). After PCR, 10 micro liter of the reaction mixture was applied to 12% polyacrylamide gel for electrophoresis and then stained with ethidium bromide. The gel was photographed and scanned using an EP Mac software. The intensities of the two bands were compared (110 bp and 87 bp) were measured using a scan analysis software. B-actin gene was used as an internal reference template to correct for the variation in quantity of the template DNA.The adjusted genomic DNA of each case and competitor DNA were simultaneously added to the PCR mixture and amplified while competing for the same EGFR primer, the reaction mixture was applied to polyacrylamide gel electrophoresis and then stained with ethedium bromide. The intensities of the bands were measured. The Ratio of EGFR product /competitor DNA PCR product (EGFR) was calculated for each case.

Results

When competitive PCR was conducted using normal oral mucosa as control, the EGFR/competitive ratio was 0.97±1.5 (mean ± standard deviation) .As a positive control, competitive PCR was performed for a model of gene amplification, using a fixed amount of the competitive DNA and 1X,2X,3X,4X the standard amount (40 ng)of the reference sample (Fig. 1a). Based on these results gene amplification was defined as an EGFR/competitor ratio higher than 1.87(1.76+0.11), EGFR amplification was observed in 3of 18 cases (17%) of ED, of 4 cases of CIS. And 4 of 20 cases (20%) of SCC (table 2). When classified by severity of dysplasia, the gene amplification was found in 0 of 2 cases of (0%) of mild ED, 1of 8 cases of (12.5%) of moderateED, and 2 of 8 cases of (25%) of severe ED.

Compared to a positive control The EGFR/competitor ratio was markedly greater than 4 fold in all 4 cases of SCC.

Discussion

In this study gene amplification had already occurred even in a case of moderate ED, this indicates that EGFR gene amplification is not only limited to SCC. In our study agene located on the same chromosome as EGFR was used as an internal reference template which rules out a false positive result due to polosomy of chromosome 7. The present study suggests that the amplification

in the early stages of dysplasia and CIS, is at a low level, the increased EGFR gene copies via amplification probably plays an important role in the development of invasive cancer.

References

1) Pindborg JJ, Reichart PA, Smith CJ, van der Waal I: Histological typing of ancer and precancer of oral mucosa.Tokyo: Springer, 1997.
2) Ishitoya J, Toriyama M, Oguchi N, Kitamura K,Ohshima M, Asano K, Yamamoto T: Gene amplification and overexpression of EGF receptor in squamous cell carcinoma of the head and neck. Br J Cancer, 59(4): 559-562, 1989.
3) Leonard JH, Kearsley JH, Chenevix-Trench G, Hayward NK: Analysis of gene amplification in head and neck sqaumous cell carcinoma. Int J Cancer, 48(4): 511-515, 1991.

Table 1. Primer sequences and sizes of PCR products obtained with genomic DNA and competitor DNA

Gene	Primer sequences	Size of PCR product
EGFR	5'-AGCCATGCCCGCATTAGCTC-3'	110 bp (Genomic)
	3'-AACCCTTCAACGTAAGGAAA-5'	87 bp (Competitor)
β-actin	5'-CTCTTTTCTTTCCCGATAGGT-3'	169 bp (Genomic)
	3'-CTCCAGCTTCTCGTAGGGTC-5'	

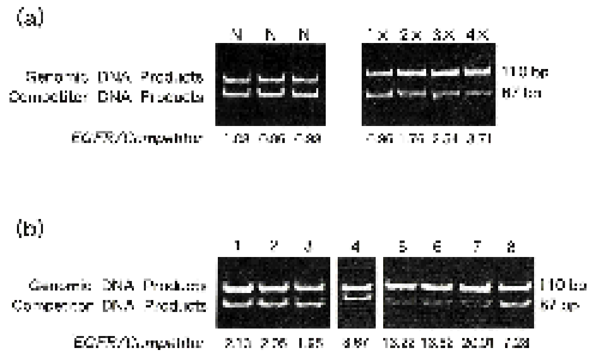

Table 2. EGFR gene amplification and degree of amplification

Histological diagnosis	No. of cases	Amplified no.(%)	Approximate degree of amplification*
Normal mucosa	6	0 (0)	
Epithelial dysplasia			
mild	2	0 (0)	
moderate	8	0 (0)	2-3×
severe	8	2 (25)	2-3×
Carcinoma in situ	4	1	4×
Squamous cell carcinoma	20	4 (20)	> 4×

* Degree of amplification in cases showing amplification