



# Abundance and localization of skeletal muscle-related erbB2 may stimulate tumour growth during initial stages of oral oncogenesis

E. Vairaktaris<sup>1\*</sup>, P. Moulavassili<sup>1\*</sup>, S. Loukeri<sup>1</sup>, S. Spyridonidou<sup>1</sup>, C. Yapijakis<sup>1,2</sup>,  
S. Vassiliou<sup>1</sup>, E. Nkenke<sup>3</sup>, A. Vylliotis<sup>1</sup>, V. Papakosta<sup>1</sup>, A. Lazaris<sup>4</sup>, E. Patsouris<sup>4</sup>

<sup>1</sup>Department of Oral and Maxillofacial Surgery, University of Athens Medical School, Athens, Greece;

<sup>2</sup>Department of Neurology, University of Athens Medical School, Eginition Hospital, Athens, Greece;

<sup>3</sup>Department of Oral and Maxillofacial Surgery, University of Erlangen, Nürnberg, Germany;

<sup>4</sup>Department of Pathology, University of Athens Medical School, Athens, Greece;

\* These authors have contributed equally to this work

## Abstract

ErbB2 and erbB3 transmembrane receptors, known to be associated with neuronal and skeletal muscle developmental function, seem to play an important role in human oral oncogenesis. This study was designed to determine gradual erbB2 and erbB3 expression in an experimental animal system of induced oral carcinogenesis in Syrian golden hamsters. Thirty-seven animals were divided into one control group ( $N=7$ ) and three experimental groups ( $N=10$  each one), which were treated with carcinogen 9,10-dimethyl-1,2-benzanthracene and sacrificed at 10, 14 and 19 weeks after treatment. The histological status of observed lesions in the three experimental groups corresponded well with tumour advancement (from oral mucosal dysplasia to moderately differentiated squamous cell carcinoma). Tissue sections ranging from normal mucosa to squamous cell carcinoma were studied using monoclonal antibodies against erbB2 and erbB3 proteins. Cytoplasmic erbB2 expression was gradually increased in pre-cancerous stages, remained stable in initial tumour stages and substantially decreased in moderately-differentiated carcinomas, suggesting that it may be useful as an early prognostic factor. On the contrary, erbB3 was not expressed at all either in normal or tumour tissue.

**Keywords:** Oral Squamous Cell Carcinoma, erbB2, erbB3, Immunohistochemistry, Histological Grade

## Introduction

Oral squamous cell carcinoma (OSCC) is one of the ten most common cancers worldwide of which 90% of them are squamous cell carcinomas<sup>1</sup>. In the last two decades an increase in incidence of oral carcinomas has been observed

The authors have no conflict of interest.

Corresponding author: Prof. Dr. Eleftherios Vairaktaris, M.D., D.D.S., Ph.D., Department of Oral and Maxillofacial Surgery, University of Athens Medical School, Vas. Sofias 93 & Dim. Soutsou 1, Athens 11521, Greece  
E-mail: lvairakt@med.uoa.gr

Accepted 30 January 2007

but the overall survival rates have not improved<sup>2</sup>. Oral oncogenesis is a multi-step process including multiple molecular genetic events in many chromosomes and genes that involve the abnormal activation of oncogenes along with the inactivation of tumour suppressor genes<sup>3</sup>. The accumulation of such genetic events seems to contribute to uncontrolled cell growth and formation of invasive carcinomas. The precise nature of the genetic alterations occurring at each step of oral carcinogenesis still remains unclear<sup>4</sup>.

Two oncogenes that are abnormally activated in OSCC are erbB2 and erbB3, which belong to the type I transmembrane tyrosine kinase family of receptors<sup>5</sup>. The ability of one c-erbB family member to influence or function synergistically with another is a general feature of these receptors<sup>5</sup>. Receptor activation through generation of ligand-dependent dimers results in

phosphorylation of specific tyrosine residues within the cytoplasmic region, which leads to the recruitment of phosphotyrosine-binding effector proteins, and subsequent stimulation of multiple signalling pathways<sup>5</sup>. In order to be activated, erbB2 and erbB3 require co-receptors, which are usually other erbBs<sup>6</sup>. ErbB2 lacks its own distinct ligand, therefore it can only take part in signal transduction pathways through association with other members of the erbB family<sup>6</sup>. ErbB3 is a receptor for heregulins (also known as neuregulins) which perform many functions during neuronal development via erbB3 receptor-mediated signalling pathways<sup>7</sup>. ErbB3 lacks an intrinsic tyrosine kinase activity, thus in order to be activated it must associate with erbB2 complementing each other's deficiency by the formation of a functional signal transduction complex<sup>6</sup>. ErbB2 is widely distributed in various tissues and shares a similar pattern of expression to that of Epidermal Growth Factor Receptor (EGFR). Interestingly, it has been detected throughout the myofibre membrane of rat skeletal muscle<sup>8</sup>. The overexpression of erbB2 in various types of cancer has been shown to promote the multiple adhesion and invasion steps of the metastatic cascade, suggesting that this gene may play an important role in carcinogenesis and metastasis<sup>9</sup>. Simultaneous expression of both erbB2 and erbB3 has been found to significantly increase their individual transforming activity, suggesting a role for erbB3 in malignant progression<sup>9</sup>.

Increased levels of the erbB2 protein have been reported in human OSCC, resulting mainly from amplification of its gene<sup>10-18</sup>. On the other hand, several studies of human OSCC have reported overexpressed erbB3 mRNA and protein but no gene amplification<sup>19-23</sup>. In tumour cells overexpressing erbB3, promoter activity is enhanced as a result of the increased abundance of the transcription factor OB2-1<sup>24</sup>. Nevertheless, there is rare information in the literature regarding the exact stages of oral oncogenesis in which overexpression of erbB2 and erbB3 actually begins.

This study was designed to determine erbB2 and erbB3 expression and their possible correlation in sequential stages of OSCC formation in an experimental animal system of induced oral carcinogenesis. In Syrian golden hamsters, the chemically induced oral pre-malignant lesions and OSCC resemble both pathologically and ultrastructurally the equivalent ones that develop in humans exposed to tobacco and alcohol<sup>25,26</sup>.

## Materials and methods

### Experimental carcinogenesis

Thirty-seven male Syrian golden hamsters (*Mesocricetus auratus*) purchased from the Hellenic Pasteur Institute (Athens) at the age of five weeks and weighed approximately 100 g each, were used in this study. The experimental protocol was approved by the General Directorate of Veterinary Services according to Greek legislation (PD 160/1991), in compliance to the EEC Directive 609/1986 and Law 2015/1992. The animals were randomly divided into four groups: one Control group (n=7) and three experimental groups for carcinogen treatment (A, B and C; n=10 animals each). The left buccal

pouches of animals in the experimental groups were treated with 0.5% 9,10-dimethyl-1,2-benzanthracene (DMBA) (Sigma, St. Louis, MO) dissolved in paraffin oil, for 10 weeks (group A) and for 14 weeks (groups B and C), as previously described<sup>27</sup>. The pouches of all animals were examined weekly in order to observe the growth of tumours on the mucosa. The treated buccal pouches were removed after sacrifice of animals at 10 weeks from application of the carcinogen (group A), at 14 weeks (group B) and at 19 weeks (group C). The Control animals were sacrificed at 10 weeks and a left buccal pouch sample was taken. The biopsies were given a number and examined blindly.

### Pathological evaluation

The histological status of the lesions was defined after examination of the complete section under light microscopy and the tissue profiles were classified in the following categories: normal, hyperkeratosis, hyperplasia, dysplasia, early invasion, well- and moderately-differentiated carcinoma. In every sample all possible different lesions were evaluated.

### Immunohistochemical analysis

The biopsies from the 37 animals were fixed in 10% neutralized formaldehyde solution and embedded in paraffin. Three sections of 4 µm were prepared from each specimen and were mounted on Super Frost Plus-coated glass slides (Menzel and Co., Braunschweig, Germany). One section was stained with hematoxylin and eosin for routine histological evaluation, while the other two were used for immunohistochemical detection of erbB2 and erbB3 proteins. The sections were incubated with monoclonal primary antibodies against c-erbB-2 (c-erbB-2/HER-2/neu Ab-17, Lab Vision Corporation, 1:150 dilution) for 30 minutes at room temperature and c-erbB-3 (C-17:sc-285, Santa Cruz Biotechnology Inc, 1:150 dilution) overnight at 4°C using standard immunohistochemical methodology, as previously described<sup>27</sup>. For erbB3 antigen retrieval was performed by incubation of the sections with trypsin for 15 minutes. Human breast carcinomas which express strongly erbB2 and erbB3 were used as positive controls. Negative controls for each biopsy were processed in the same manner, using Phosphate Buffer Saline (PBS) instead of the primary antibody. All slides were independently reviewed by two investigators blindly. The consecutive hematoxylin-eosin-stained slides were evaluated by a pathologist experienced in oral pathology, without knowing the erbB2 and erbB3 staining patterns.

### Statistical analysis

The mean value of percentages of positive stained cells was calculated from all the different lesions present in each sample. These values were tabulated for each group of animals (control group, experimental groups A, B, C) and were compared for every pair of sequential groups.

In order to evaluate the pattern of antibody expression in relation to the histological status, the various lesions were

	Control group	Group A	Group B	Group C
Normal tissue	4			
Hyperkeratosis	2			
Hyperplasia	1			
Dysplasia		6		
Early invasion		3	1	
Well differentiated carcinoma		1	6	1
Moderately differentiated carcinoma			3	9

**Table 1.** Histological status of biopsies in the control group and in the three experimental groups.

	Normal oral mucosa	Oral mucosa with hyperkeratosis	Oral mucosal hyperplasia	Oral mucosal dysplasia	Early invasion	Tumour	
						Well- differentiated OSCC	Moderately- differentiated OSCC
<b>a. General</b>							
Mean percentage (%)	6.7 (N=15)	42 (N=5)	56.3 (N=16)	64.1 (N=22)	78.4 (N=19)	82.5 (N=24)	45.6 (N=25)
Mean (%) of pre-cancerous conditions		58.6					
T-test probability		<b>P&lt;0.05</b>		<b>P&lt;0.001</b>	<b>P&lt;0.001</b>	<b>P&lt;0.05</b>	<b>P&lt;0.01</b>
<b>b. Membrane</b>							
Mean percentage (%)	0.3 (N=15)	54 (N=5)	57.8 (N=16)	41.4 (N=22)	48.7 (N=19)	22.3 (N=24)	19.7 (N=25)
Mean (%) of pre-cancerous conditions		49					
T-test probability		<b>P&lt;0.001</b>		<b>P&lt;0.001</b>	<b>P&lt;0.001</b>	N.S	<b>P&lt;0.01</b>
<b>c. Cytoplasmic</b>							
Mean percentage (%)	6.7 (N=15)	42 (N=5)	80 (N=16)	90.5 (N=22)	91.6 (N=19)	88.8 (N=24)	74.8 (N=25)
Mean (%) of pre-cancerous conditions		80.9					
T-test probability		<b>P&lt;0.05</b>		<b>P&lt;0.001</b>	<b>P&lt;0.001</b>	N.S	N.S

**Table 2.** Percentages of erbB2 positive cells in the various categories of tissue status and in regard to localization of staining: a) general (both membrane and cytoplasm) b) membrane only c) cytoplasm only. N.S.: No statistical difference.

divided according to tumour progression in a) normal tissue, b) non-cancerous and pre-cancerous conditions (hyperkeratosis, hyperplasia, dysplasia), c) tumour (early invasion, well-differentiated carcinoma, moderately-differentiated carcinoma). In every lesion the percentages of positively stained cells exhibiting membrane or cytoplasmic staining were also calculated. The percentages of positively stained cells (general), membrane positively stained cells (membrane) and cytoplasmic positively stained cells (cytoplasmic) from each non-cancerous and precancerous category were compared with those of normal tissue, while the percentages of positively stained cells from each tumour category were compared with the mean percentage of the two non-cancerous and one pre-cancerous lesions.

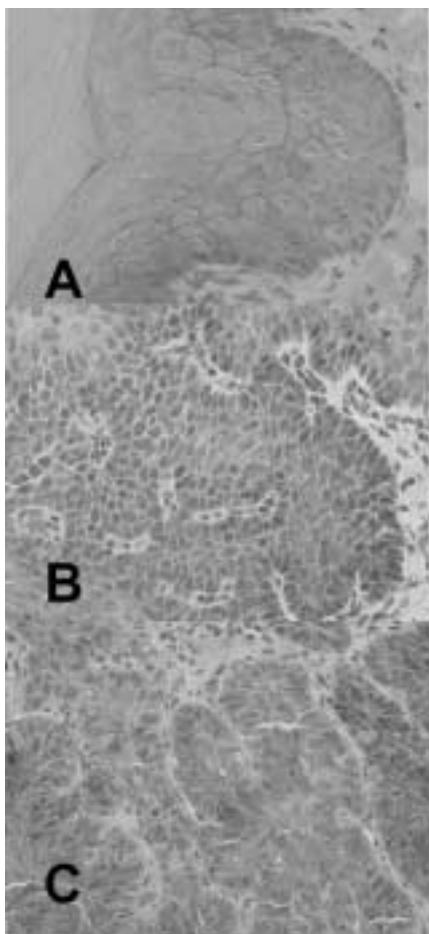
A two-tailed student's t-test was applied for statistical analysis using the SPSS 10.0 program for Windows™. In addition, in every group and every histological category a nor-

mal distribution check was performed using the Kolmogorov-Smirnov Z test of SPSS. If a group or a histological category was not normally distributed, additional statistical analysis was performed with the Wilcoxon test using SPSS.

## Results

A progression towards OSCC formation in correlation to the increased time of carcinogenesis was evident by the histological status of biopsies in the four studied groups of animals (Table 1). Therefore, as expected, this experimental model seems valid and further analysis of immunostaining data was implemented. In all cases with no normal distribution, the results of both the Wilcoxon test and two-tailed student's t-test provided the same level of significance.

Cell membrane staining of erbB2 in hyperkeratotic tissues



**Figure 1.** Immunohistochemical staining of oral tissues using erbB2 antibody is shown: (A) erbB2 + (5-20% positive cells) cell membrane stained hyperplastic oral mucosa; (B) erbB2 ++ (20-50% positive cells) cytoplasmic staining of oral mucosa well-differentiated carcinoma; (C) erbB2 +++ (>50% positive cells) cytoplasmic and cell membrane staining of oral mucosa early invasion,  $\times 40$ .

was found to be increased compared to normal ones (Table 2). A stable pattern of erbB2 expression was observed in the following non-cancerous, pre-cancerous and early tumour stages, followed by a statistically significant decrease in the last two observed cancer stages (well-differentiated and moderately-differentiated carcinoma) of this particular experimental system (Table 2). Interestingly, cytoplasmic staining exhibited a different pattern of erbB2 expression. The percentage of positively stained cells rose gradually from normal to non-cancerous and pre-cancerous tissues, while a relatively stable expression was observed in the following tumour stages, with a slight non-significant decrease in well-differentiated carcinomas (Figure 1). Another interesting difference is that in cancerous conditions the percentage of cells exhibiting cytoplasmic staining is remarkably elevated compared to the equivalent one in cells expressing erbB2 in cell membrane.

A similar pattern of initial increase and stabilization of erbB2 expression was revealed by statistical analysis performed for the control group and the three experimental groups (Table 3). The percentage of erbB2 positively stained cells was found to be increased in group A compared to the control group ( $P < 0.05$ ). An additional increase in group B, followed by a slight decrease of erbB2 expression was not statistically significant.

The immunohistochemical analysis performed for c-erbB-3 antibody in normal, pre-cancerous and cancer tissues revealed a complete absence of expression.

## Discussion

In this study, the expression of erbB2 during various stages of oral oncogenesis was evaluated in an experimental system. A gradual increase of erbB2 expression from normal oral mucosa to non-cancerous and pre-cancerous stages was observed. In the initial tumour stages the percentages of positively erbB2 stained cells remained almost stable, while in the stage of moderately-differentiated carcinoma a substantial decrease was observed. Interestingly, the observed erbB2 cytoplasmic staining was very elevated compared to membrane staining in the initial stages of oral oncogenesis, while both of them decreased during the later stages of oral oncogenesis.

The findings of this study indicate the abundance of cytoplasmic erbB2 in oral carcinomas. It is known that the extracellular region of erbB2 can be proteolytically removed into the sera of patients with malignancies of an advanced stage (such as breast carcinoma) and therefore the proportion of receptors attached in the cell membrane is substantially eliminated<sup>28</sup>. In addition, in tumour patients the glucose-chaperone protein GRP94 forms stable complexes with overexpressed erbB2 resulting in its proteolysis. As a result, this association does not permit the localization of erbB2 in the membrane<sup>29</sup>. Furthermore, the abundance of erbB2 in cytoplasm may also be attributed to alternative RNA processing which produces secreted forms of c-erbB2 that do not contain the transmembrane regions resulting in its cytoplasmic localization<sup>30</sup>.

ErbB2 is a receptor without its own distinct ligand, thus in order to be activated it has to associate with other erbBs<sup>5,6</sup>. In this experimental study, abundance and cytoplasmic localization of erbB2 was observed indicating a close relationship with carcinogenesis, since overexpression of erbB2 leads to its increased association with other erbBs and to increased activation of the signal transduction pathways affecting cellular growth, division and differentiation<sup>5</sup>.

The findings of this study are in agreement with previous reports of humans where pre-malignant and malignant oral lesions showed progressive increase of erbB2 expression as the cells acquired a more malignant phenotype<sup>16,17</sup>. Nevertheless, previous reports did not perform a detailed analysis of membrane or cytoplasmic erbB2 expression in all possible stages of oral oncogenesis. In addition, several other reports have detected high levels of erbB2 expression when

<i>c-erbB-2</i>	<b>Control Group (N=7)</b>	<b>Group A (N=10)</b>	<b>Group B (N=10)</b>	<b>Group C (N=10)</b>
<b>Mean value (%) of percentages</b>	20.2	61.4	75	57.7
<b>Probability of t-test</b>		<b>P&lt;0.05</b>	N.S.	N.S.

**Table 3.** Percentages of erbB2 positive cells in the control group and three experimental groups. N.S.: No statistical difference.

compared to normal or benign oral lesions although a classification of their results according to the progression of oral cancer had not been performed<sup>12,14,21</sup>.

On the other hand, this study revealed no immunoreactivity of erbB3 in contrast to several reports which have observed overexpression of erbB3 in human OSCC compared to normal oral mucosal tissue<sup>19-23</sup>. Although erbB3 has already been studied and analysed in experimental murine models<sup>31</sup>, it has never before been studied in hamsters and its gene remains unknown. It is possible that this particular animal lacks erbB3 and its role of signal transduction in normal or tumour oral tissue is undertaken by another erbB factor.

## Conclusions

In conclusion, this study is the first one regarding analysis of erbB2 and erbB3 expression in an experimental animal system of induced gradual oral carcinogenesis. This experimental system in hamsters indicates a strong correlation between erbB2 overexpression and oral cancer progression and an absence of erbB3 expression in normal or tumour tissues. The findings of this study regarding erbB2 may be used as an early prognostic marker in human oral cancer. If erbB2 overexpression is detected in pre-cancerous oral lesions such as leukoplakia, it seems that the best strategy for cancer prevention is immediate surgical treatment.

## Acknowledgements

This work was co-funded by the European Social Fund and National Resources (EPEAEK II "Pythagoras" 70/3/7391) grant to E.V.

## References

- 1. Hardisson D. Molecular pathogenesis of head and neck squamous cell carcinoma. Eur Arch Otorhinolaryngol 2003; 260:502-508.
- 2. Reichart PA. Identification of risk groups for oral pre-cancer and cancer and preventive measures. Clin Oral Invest 2001; 5:207-213.
- 3. Scully C, Field JK, Tanzawa H. Genetic aberrations in oral or head and neck squamous cell carcinoma (SCCHN): 1. Carcinogen metabolism, DNA repair and cell cycle control. Oral Oncol 2000; 36:256-263.
- 4. Nagpal JK, Das BR. Oral cancer: reviewing the present understanding of its molecular mechanism and exploring the future directions for its effective management. Oral Oncol 2003; 39:213-221.
- 5. O-Charoenrat P, Rhys-Evans PH, Modjtahedi H, Eccles S. The role of c-erbB receptors in head and neck squamous cell carcinoma. Oral Oncol 2002; 38:627-640.
- 6. Nagy P, Jenei A, Damjanovich S, Jovin TM, Szollosi J. Complexity of signal transduction mediated by erbB2: clues to the potential of receptor-targeted cancer therapy. Pathol Oncol Res 1999; 5:255-271.
- 7. Buonanno A, Fischbach GD. Neuregulin and erbB receptor signalling pathways in the nervous system. Curr Opin Neurobiol 2001; 11:287-296.
- 8. Altiok N, Beessereau JL, Changeux JP. ErbB3 and erbB2/neu mediate the effect of heregulin on acetylcholine receptor gene expression in muscle: differential expression at the endplate. EMBO J 1995; 14:4258-4266.
- 9. Yu D, Wang SS, Dulski MM, Tsai SM, Nicolson GL, Hung MC. C-erbB-2/neu overexpression enhances metastatic potential of human lung cancer cells by induction of metastasis-associated properties. Cancer Res 1994; 54:3260-3266.
- 10. O-Charoenrat P, Rhys-Evans PH, Eccles S. Characterization of ten newly-derived human head and neck squamous carcinoma cell lines with special reference to c-erbB proto-oncogene expression. Anticancer Res 2001; 21:1953-1963.
- 11. Werkmeister R, Brandt B, Joos U. Clinical relevance of erbB-1 and -2 oncogenes in oral carcinomas. Oral Oncol 2000; 36:100-105.
- 12. Werkmeister R, Brandt B, Joos U. The erbB oncogenes as prognostic markers in oral squamous cell carcinomas. Am J Surg 1996; 172:681-683.
- 13. Nagler RM, Kerner H, Laufer D, Ben-Eliezer S, Minkov I, Ben-Itzhak O. Squamous cell carcinoma of the tongue: the prevalence and prognostic roles of p53, Bcl-2, c-erbB-2 and apoptotic rate as related to clinical and pathological characteristics in a retrospective study. Cancer Lett 2002; 186:137-150.
- 14. Albuquresque RLC Jr, Miguel MCC, Costa ALL, Souza LB. Correlation of c-erbB-2 and S-100 expression with the malignancy grading and anatomical site in oral squamous cell carcinoma. Int J Exp Pathol 2003; 84:259-265.
- 15. Khademi B, Shirazi FM, Vasei M, Doroudchi M, Gandomi B, Modjtahedi H, Pezeshki AM, Ghaderi A. The expression of p53, c-erbB-1 and c-erbB-2 molecules

- and their correlation with prognostic markers in patients with head and neck tumours. *Cancer Lett* 2002; 184:223-230.
16. Xia W, Lau YK, Zhang HZ, Xiao FY, Johnston DA, Liu AR, Li L, Katz RL, Hung MC. Combination of EGFR, HER-2/neu, and HER-3 is a stronger predictor for the outcome of oral squamous cell carcinoma than any individual family members. *Clin Cancer Res* 1999; 5:4164-4174.
  17. Hou L, Shi D, Tu SM, Zhang HZ, Hung MC, Ling D. Oral cancer progression and c-erbB-2/neu proto-oncogene expression. *Cancer Lett* 1992; 65:215-220.
  18. Wilkman TS, Hietanen JH, Malmstrom MJ, Konttinen YT. Immunohistochemical analysis of the oncoprotein c-erbB-2 expression in oral benign and malignant lesions. *Int J Oral Maxillofac Surg* 1998; 27:209-212.
  19. Shintani S, Funayama T, Yoshihama Y, Alcalde RE, Matsumura T. Prognostic significance of erbB3 overexpression in oral squamous cell carcinoma. *Cancer Lett* 1995; 95:79-83.
  20. Funayama T, Nakanishi T, Takahashi K, Taniguchi S, Takigawa M, Matsumura T. Overexpression of c-erbB-3 in various stages of human squamous cell carcinomas. *Oncology* 1998; 55:161-167.
  21. Issing WJ, Heppt WJ, Kastenbauer ER. ErbB-3, a third member of the erbB/epidermal growth factor receptor gene family: its expression in head and neck cancer cell lines. *Eur Arch Otorhinolaryngol* 1993; 250:392-395.
  22. Bei R, Pompa G, Vitolo D, Moriconi E, Ciocci L, Quaranta M, Frati L, Kraus MH, Muraro R: Co-localization of multiple erbB receptors in stratified epithelium of oral squamous cell carcinoma. *J Pathol* 2001; 195:343-348.
  23. De Vicente JC, Esteban I, Germana P, Germana A, Vega JA. Expression of erbB3 and erbB4 protooncogene proteins in oral squamous cell carcinoma: a pilot study. *Med Oral* 2003; 8:374-381.
  24. Skinner A, Hurst HC. Transcriptional regulation of the c-erbB-3 gene in human breast carcinoma cell lines. *Oncogene* 1993; 8:3393-3401.
  25. Chang KW, Saraj S, Lin SC, Tsai PI, Solt D. p53 expression p53 and Ha-ras mutation and telomerase activation during nitrosamine-mediated hamster pouch carcinogenesis. *Carcinogenesis* 2000; 21:1441-1451.
  26. Balasenthil S, Saroja M, Ramachandran CR, Nagini S. Of humans and hamsters: comparative analysis of lipid peroxidation, glutathione and glutathione-dependent enzymes during oral carcinogenesis. *Br J Oral Maxillofac Surg* 2000; 38:267-270.
  27. Derka S, Vairaktaris E, Papakosta V, Vassiliou S, Acil Y, Vyliotis A, Spyridonidou S, Lazaris AC, Mourouzis C, Kokkori A, Moulavassili P, Perrea D, Donta I, Yapijakis C, Patsouris E. Cell proliferation and apoptosis culminate in early stages of oral oncogenesis. *Oral Oncol* 2006; 42:540-550.
  28. Pupa SM, Menard S, Morelli D, Pozzi B, De Palo G, Colnaghi MI. The extracellular domain of the c-erbB-2 oncoprotein is released from tumour cells by proteolytic cleavage. *Oncogene* 1993; 8:2917-2923.
  29. Chavany C, Mimnaugh E, Miller P, Bitton R, Nguyen P, Trepel J, Whitesell L, Schnur R, Moyer J, Neckers L. p185erbB2 binds to GRP94 *in vivo*. *J Biol Chem* 1993; 271:4974-4977.
  30. Scott GK, Robles R, Park JW, Montgomery PA, Daniel J, Holmes WE, Lee J, Keller GA, Li Wen-L, Fendly BM, Wood WI, Shepard HM, Benz CC. A truncated intracellular HER2/neu receptor produced by alternative RNA processing affects growth of human carcinoma cells. *Mol Cell Biol* 1993; 13: 2247-2257.
  31. Troyer KL, Lee DC. Regulation of mouse mammary gland development and tumorigenesis by the ERBB signalling network. *J Mammary Gland Biol Neoplasia* 2001; 6:7-21.