# Involvement of EphA2 in head and neck squamous cell carcinoma: mRNA expression, loss of heterozygosity and immunohistochemical studies

ROSARIO S. RIVERA[1,2], MEHMET GUNDUZ[1,4], HITOSHI NAGATSUKA[1], ESRA GUNDUZ[1], BEYHAN CENGIZ[1], KUNIHIRO FUKUSHIMA[3], LEVENT BEKIR BEDER[4], DAVUT PEHLIVAN[5], NOBORU YAMANAKA[4], KENJI SHIMIZU[6] and NORIYUKI NAGAI[1]

[1]Department of Oral Pathology and Medicine, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Okayama City, Japan; [2]University of the East, College of Dentistry, Manila, Philippines; [3]Otolaryngology, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Okayama City; [4]Department of Otolaryngology, Wakayama Medical University, Kimiidera, Japan; [5]Department of Medical Genetics, Istanbul University, Istanbul Medical Faculty, Turkey; [6]Molecular Genetics, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Okayama City, Japan

Received December 4, 2007; Accepted January 9, 2008

**Abstract.** EphA2 is a 130-kDa transmembrane protein primarily found in adult human epithelial cells and is a member of one of the largest receptor tyrosine kinases. It is located on 1p36.1, a genetic hot spot in cancer. EphA2 overexpression has been observed in aggressive solid tumors and its potential role in tumorigenesis, which includes cell growth, survival, migration and angiogenesis have been reported. However, the role of EphA2 remains unknown in head and neck cancer. In this study, we investigated the genetic profile of EphA2 in primary head and neck squamous cell carcinoma (HNSCC) by determining mRNA level, status of loss of heterozygosity and protein expression. mRNA expression was also correlated with clinicopathological data. Infrequent loss of heterozygosity (20%) was observed, though a 10-fold increase of mRNA expression in tumors compared to normal tissues was noted. A significant number of samples with normal to high mRNA expression was observed among patients with regional metastasis, with T3-T4 tumor size and with moderate to poor differentiation. However, statistical studies did not show any correlation between mRNA expression and any of the clinicopathological parameters. Tumor cells expressed EphA2 protein, but only weakly. These results suggest that EphA2 might be involved in the early development of HNSCC although not directly responsible for its progression.

*Correspondence to*: Dr Mehmet Gunduz, Department of Oral Pathology and Medicine, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, 2-5-1 Shikata-cho, Okayama City 700-8528, Japan
E-mail: mgunduz@md.okayama-u.ac.jp

*Key words:* EphA2, head and neck squamous cell carcinoma, mRNA expression, loss of heterozygosity, immunohistochemistry

## Introduction

The Eph family is one of the largest receptor tyrosine kinases (RTK) in which their ligand (ephrin) binds via direct cell-to-cell contact (1,2). At least 14 members of the Eph family have been recognized and are divided into EphA and EphB depending on the type of interaction with their ligands. Unlike the other members, EphA2 is expressed in adult epithelial tissues (2-5). The cellular functions of EphA2 in normal epithelium are not well understood although studies on tumor-based models suggested the potential role of EphA2 in cell growth, survival, migration and angiogenesis (6,7). Furthermore, EphA2 is located in chromosome 1p36.1, which is identified as a genetic hot spot in cancer since many tumor suppressor genes (TSG) and proto-oncogenes are known to reside in this segment (8,9). This segment is deleted in certain cancers though amplified in others (8-11). Genetic changes could then lead to either the inactivation of a TSG or the activation of proto-oncogene, contributing to the pathogenesis of the tumor (9).

Although overexpression of EphA2 has been implicated in aggressiveness, poor prognosis and metastasis in various tumors, the genetic profile of EphA2 in head and neck squamous cell carcinoma (HNSCC) has not yet been characterized (12-20). The pattern of inactivation or activation of EphA2 may provide insight into its role in the tumorigenesis of HNSCC. Therefore in the current study, we investigated the loss of heterozygosity (LOH), mRNA expression and protein expression of EphA2 in HNSCC. The mRNA level was also correlated with clinicopathological data.

## Materials and methods

*Tissue samples*. Paired normal and tumor samples were obtained from the Department of Otolaryngology, Okayama University Hospital with written consent from the patients. Tissue samples consisted of normal and tumor tissues from surgical specimens of 77 patients diagnosed as primary SCC of the head and neck region. The surgical specimens were immediately frozen after surgery in liquid nitrogen and stored at -80˚C prior to DNA and RNA extractions. A histological examination was performed in the Department of Pathology and every tumor was confirmed as SCC. The study was approved by the Institutional Review Board of our University.

*DNA and RNA extraction*. Briefly, genomic DNA and total RNA were isolated from frozen tissues by SDS/proteinase K treatment, phenol-chloroform extraction and ethanol precipitation as described previously (21,22).

*cDNA synthesis*. cDNA was synthesized by reverse transcription using Rever Tran Ace α (Toyobo Co., Ltd., Osaka, Japan) in a 20 $\mu$l mixture of 4 $\mu$l 5x buffer, 2 $\mu$l dNTP, 1 $\mu$l Oligo dT Primer, 1 $\mu$l RNase inhibitor, 1 $\mu$l reverse transcriptase, 1 $\mu$g/$\mu$l total RNA and RNase free water. Samples were incubated at 30˚C for 10 min, then at 42˚C for 20 min and at 99˚C for 5 min. Samples were then immediately chilled on ice and collected by spinning down.

*mRNA expression*. mRNA expression was analyzed in paired normal and tumor cDNA samples using forward (5'-CAA GAC CCT GGC TGA CTT TGA C) and reverse (5'-AGT TGC AGG GGG AGG AAA GAA C) primers, designed using GENETYX-MAC 10.1 (Software Development Co., Ltd., Tokyo, Japan) based on the nucleotide sequence NM_004431. RT-PCR was performed in 20 $\mu$l reactions containing 2 $\mu$l reaction buffer, 1.6 $\mu$l dNTP, 0.5 $\mu$l primer, 0.1 $\mu$l rTaq (Takara Bio Inc., Shiga, Japan) and diluted to volume with sterile water. RT-PCR cycling conditions used were: initial denaturation at 94˚C for 3 min, 35 cycles (in some cases, 38 cycles were used due to insufficient amplification) of denaturation at 94˚C for 30 sec, annealing at 60˚C for 1 min, and extension at 72˚C for 1 min and then a final extension at 72˚C for 1 min. Twenty-five cycles for RT-PCR of GAPDH expression using primers, forward (5'-AGA CCA CAG TCC ATG CCA TCA C) and reverse (5'-GGT CCA CCA CCC TGT TGC TGT) primers were performed under the same conditions.

The density of the bands from the RT-PCR products was measured using ImageJ 1.38 (http://rsb.info.nih.gov/ij). The value of tumor-specific EphA2 expression was determined by calculating the ratio of the expression level in the tumor and that of the matched normal sample, normalized to the corresponding GAPDH expression level (T = EphA2/GAPDH expression ratio in tumor samples; N = EphA2/GAPDH expression ratio in matched normal sample; T/N ratio was the relative EphA2 expression in the tumor sample). Decreased and increased expression levels were defined when the ratio was 0.5 (a decrease in 50% or more) and 1.5 (an increase in 50% or more), respectively. The expression level between 0.5 and 1.5 was considered normal. Reproducibility was confirmed by a second independent RT-PCR.

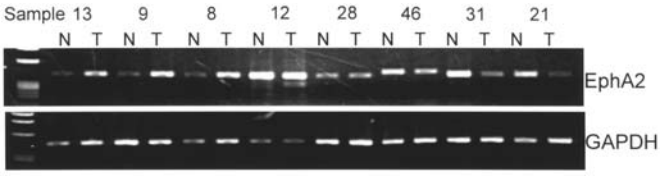


Figure 1. EphA2 mRNA expression. Representative samples of mRNA expression from paired normal and tumor samples. Note the increase in intensity of EphA2 mRNA expression in the tumor samples compared to the normal. After normalization with GAPDH, samples 13, 9 and 8 yielded high, samples 12, 28 and 46 yielded normal and samples 31 and 21 yielded low EphA2 mRNA expression.

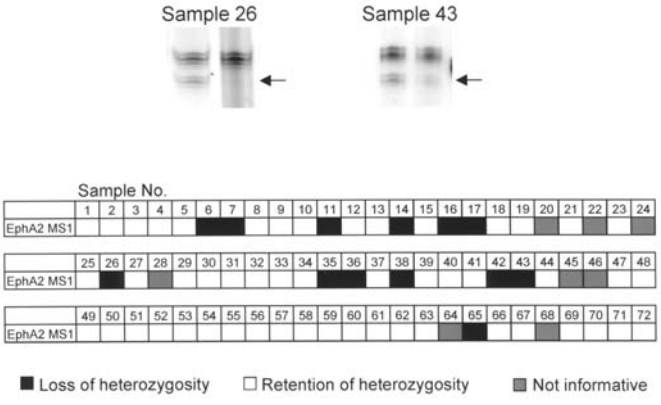


Figure 2. LOH analysis. Representative samples of cases with LOH (arrows). Above is a schematic representation of the LOH analysis in 72 samples.

*LOH analysis*. LOH analysis was performed on paired 72 normal and tumor genomic DNA samples using EphA2-specific microsatellite EphA2 MS1 forward (5'-TCC TGT CAT CCT GTC TCT AT) and reverse (5'-TAC AGT GGT GCG ATC TTG G) primers. The mapping information and sequences were obtained from the recent genome information (http://www.ncbi.nlm.nih.gov/genome/guide/human). The heterozygosity and repeat number of the tandem nucleotide repeats for the design of microsatellite marker were acquired from the information site (http://www.gramene.org/db/searches/ssrtool) (23). The primers were designed based on the contiguous genomic sequence (NM_004873) using GENETYX-MAC 10.1. The sequence of the primers was located at 90 kbp telomere side of EphA2, detecting the status of the allelic loss of the EphA2 gene.

PCR was performed in 20 $\mu$l reactions containing 2 $\mu$l reaction buffer, 1.6 $\mu$l dNTP, 0.5 $\mu$l primer, 0.1 $\mu$l rTaq (Takara Bio Inc.) and diluted to volume with sterile water. PCR cycling conditions used were: initial denaturation at 94˚C for 3 min, 25 cycles of denaturation at 94˚C for 30 sec, annealing at 58˚C for 1 min and extension at 72˚C for 1 min and a final extension at 72˚C for 1 min. PCR products were then loaded in 8% polyacrylamide gel electrophoresis and DNA bands were visualized by silver staining as described previously (21,22). LOH was scored if one of the

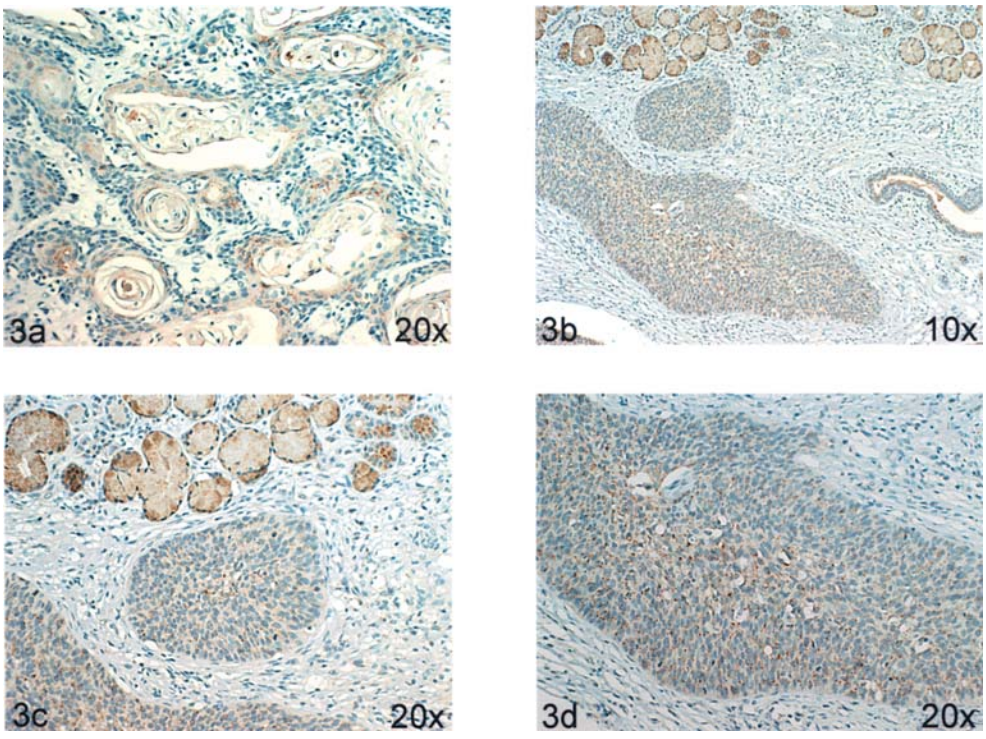


Figure 3. EphA2 protein expression. Tumor cells weakly expressed EphA2. (a) Note that only the tumor cells adjacent to the central keratinization expressed EphA2. (b and c) The intensity of the expression of EphA2 by the tumor cells and adjacent salivary glands. (c) Note that the expression in the adjacent salivary glands is more intense than that of the tumor cells. (d) EphA2 expression was noted at the cell surface and within the cytoplasm.

heterozygous alleles of the tumor DNA showed at least a 50% reduction in the intensity compared to the normal DNA. LOH frequency was obtained by dividing the total number of LOH samples with the total number of informative samples. LOH frequency rate of >30% was considered high.

*Immunohistochemistry (IHC)*. Paraffin sections of 4 $\mu$m were sectioned from chosen blocks. Slides were deparaffinized, dehydrated and then immersed in 0.3% hydrogen peroxide-methanol for 30 min. The slides were treated with citrate buffer, pH 6.0, in a pressure cooker for 10 min. Primary monoclonal EphA2 antibody (clone D7, Upstate Biotechnology, Inc., Lake Placid, NY) was used with a dilution of 1:100 and was incubated overnight at 4˚C. Detection of immunoreaction was done with 3,3'-diaminobenzidine and counterstained with Mayer's hematoxylin.

*Statistical analysis*. The correlation between mRNA level and clinicopathological data were statistically analyzed using the Student's t-test (for age) and the Fisher exact probability test. P-values <0.05 were considered significant.

## Results

*mRNA expression*. After normalization with GAPDH, mRNA expression levels were quantified using computer-based software and were normalized with GAPDH as described above. A high expression was obtained in 26% (20/77; mean value = 4.12) and a normal expression was obtained in 40% (31/77; mean value = 0.91), and a low expression was obtained in 34% (26/77; mean value = 0.29) (Table I). Fig. 1 shows EphA2 mRNA expression in normal and tumor samples compared with GAPDH.

*LOH analysis*. To determine the possible occurrence of EphA2 inactivation in HNSCC, LOH analysis was performed using an EphA2-specific microsatellite marker. Only 20% (13/64) of the samples demonstrated LOH (Fig. 2).

*Immunohistochemistry*. Samples with normal and high expression levels were selected for IHC analysis. EphA2 expression was focal and weak compared to the intensity of the normal epithelium and the surrounding normal salivary glands. The expression was mostly noted at the cell membrane or within the cytoplasm (Fig. 3).

*Comparison of clinicopathological data with EphA2 mRNA expression*. The mRNA level was correlated with the clinicopathological data (Table II). Samples were grouped based on the level of expression, i.e. low and normal to high. Only 61 samples were included in the analysis due to incomplete parameters of the other 16 samples. One notable result was a possible correlation between regional metastasis and EphA2 mRNA expression with a nearly statistically significant data. Although 19% (4/21) of the patients with EphA2 down-regulation showed regional metastasis, 43% (17/40) of the patients with normal to high EphA2 mRNA expression revealed regional metastasis suggesting the possible involvement of EphA2 in local invasion of HNSCC.

Moreover, a significant number of patients with T3-T4 tumor size and those with moderate to poor differentiation

Table I. EphA2 mRNA expression.

| Sample | Value | Expression | Sample | Value | Expression | Sample | Value | Expression |
|---|---|---|---|---|---|---|---|---|
| 1 | 1.85 | H | 27 | 0.88 | N | 53 | 4.16 | H |
| 2 | 1.87 | H | 28 | 1.09 | N | 54 | 0.59 | N |
| 3 | 0.69 | N | 29 | 2.49 | H | 55 | 0.4 | L |
| 4 | 0.74 | N | 30 | 0.13 | L | 56 | 3.28 | H |
| 5 | 0.44 | L | 31 | 0.42 | L | 57 | 0.17 | L |
| 6 | 0.46 | L | 32 | 0.56 | N | 58 | 0.32 | L |
| 7 | 1.02 | N | 33 | 10.86 | H | 59 | 7.59 | H |
| 8 | 4.2 | H | 34 | 0.72 | N | 60 | 0.68 | N |
| 9 | 5.23 | H | 35 | 0.46 | L | 61 | 4.9 | H |
| 10 | 1.13 | N | 36 | 3.84 | H | 62 | 1.42 | N |
| 11 | 0.99 | N | 37 | 0.01 | L | 63 | 0.2 | L |
| 12 | 1.39 | N | 38 | 2.84 | H | 64 | 0.39 | L |
| 13 | 2.72 | H | 39 | 0.28 | L | 65 | 0.96 | N |
| 14 | 0.4 | N | 40 | 0.27 | L | 66 | 0.19 | L |
| 15 | 6.81 | H | 41 | 0.92 | N | 67 | 0.49 | L |
| 16 | 1.52 | H | 42 | 1.04 | N | 68 | 0.03 | L |
| 17 | 9.08 | H | 43 | 0.41 | L | 69 | 0.97 | N |
| 18 | 0.22 | L | 44 | 1.19 | N | 70 | 0.77 | N |
| 19 | 0.23 | L | 45 | 0.68 | N | 71 | 0.74 | N |
| 20 | 0.35 | L | 46 | 0.56 | N | 72 | 0.23 | L |
| 21 | 0.46 | L | 47 | 0.71 | N | 73 | 3.73 | H |
| 22 | 0.29 | L | 48 | 1.05 | N | 74 | 0.82 | N |
| 23 | 1.52 | H | 49 | 1.43 | N | 75 | 0.24 | L |
| 24 | 1.92 | H | 50 | 0.73 | N | 76 | 2.08 | H |
| 25 | 0.79 | N | 51 | 1.11 | N | 77 | 1.08 | N |
| 26 | 0.64 | N | 52 | 0.09 | L | | | |

H, high; N, normal; L, low.

exhibited normal to high mRNA expression. However, no correlation was observed with any of the parameters after statistical analysis.

## Discussion

Squamous cell carcinoma is a common malignant epithelial tumor in the head and neck region. In spite of advancements in treatment, HNSCC poses a major enigma with 51% regional metastasis and 10% distant metastasis upon presentation (24,25). Characterization of the EphA2 genetic profile may contribute to the understanding of the molecular therapy of HNSCC. EphA2 overexpression has been implicated in tumorigenesis of various cancers as well as cell lines. Its role was not limited simply as a marker but rather as an active participant in malignant progression (20,26). In this study, we characterized the mRNA expression and LOH frequency of EphA2 in HNSCC in order to determine whether EphA2 contributes to the malignant character of HNSCC brought about by genetic changes.

EphA2 is located in 1p36.1 and chromosomal alteration involving this region has been reported in several tumors implicating the presence of TSG (8,27). Although EphA2 overexpression was reported previously implicating it as a possible oncogene, LOH was also evaluated to determine the possibility of inactivation. However, low LOH frequency was observed suggesting rare inactivation of EphA2 and that, it does not function as a TSG in HNSCC.

The increase in EphA2 mRNA level in tumor samples compared to normal samples suggests the activation of EphA2 making it a candidate oncogene in HNSCC. The mean value of samples with normal and high expression was almost ten times than that of the low expression. This concurs with previous studies, which showed increased EphA2 expression in tumors (12,18,26). Several mechanisms have been implicated in its up-regulation. EphA2 is regulated by p53 through a response element on EphA2's promoter which is involved in cell survival (28). Ras as well as c-myc signaling pathways have also been implicated in the up-regulation of EphA2 (20,29), suggesting early regulation of EphA2 in HNSCC tumorigenesis.

Parameters such as smoking, alcohol intake and lymph node involvement have been widely reported to be associated with the progression of cancers. In this study, normal to high expression levels were observed among patients with regional metastasis, those with T3-T4 tumor size as well as those with moderate to poorly differentiated samples. A trend was seen between mRNA level and that of regional metastasis

Table II. Correlation between EphA2 mRNA expression and clinicopathological data.

| Parameter | Low expression (n=21) | Normal to high expression (n=40) | P-value |
|---|---|---|---|
| [a]Age | | | |
| Mean | 65±7.49 | 64.9±9.23 | 0.95 |
| Gender | | | |
| Male | 18 | 33 | |
| Female | 3 | 7 | 1.00 |
| Smoking | | | |
| Positive | 16 | 30 | |
| Negative | 5 | 10 | 1.00 |
| Alcohol intake | | | |
| Positive | 10 | 22 | |
| Negative | 11 | 18 | 0.60 |
| Tumor size | | | |
| T1-T2 | 10 | 13 | |
| T3-T4 | 11 | 27 | 0.28 |
| Nodal involvement | | | |
| Positive | 13 | 20 | |
| Negative | 8 | 20 | 0.43 |
| Cancer history | | | |
| Positive | 4 | 6 | |
| Negative | 17 | 34 | 0.72 |
| Family cancer history | | | |
| Positive | 10 | 18 | |
| Negative | 11 | 22 | 1.00 |
| Local recurrence | | | |
| Positive | 5 | 12 | |
| Negative | 16 | 28 | 0.77 |
| Histological differentiation | | | |
| Well | 9 | 11 | |
| Moderate to poor | 12 | 29 | 0.26 |
| Regional metastasis | | | |
| Positive | 4 | 17 | |
| Negative | 17 | 23 | 0.09 |
| Distant metastasis | | | |
| Positive | 6 | 7 | |
| Negative | 15 | 33 | 0.34 |

[a]Student's t-test.

suggesting the possible involvement of EphA2 mRNA over-expression in regional metastasis. Nevertheless, no significant correlation was obtained between mRNA expression and any of the clinicopathological parameters suggesting that EphA2 may not be a major contributor to the progression of HNSCC.

EphA2 protein overexpression has also been observed in many tumors (12-20). However, our IHC analysis revealed weak and sometimes focal expression of EphA2. High levels of EphA2 can arise in tumor cells as a result of increased protein stability (26). In contrast to other RTK, ligand binding is not necessary for tyrosine kinase activity of EphA2 and it remains unphosphorylated and accumulates in the cell surface. Furthermore, overexpression of low molecular weight tyrosine phosphatase (LMW-PTP) down-regulated the phosphotyrosine content of EphA2, which caused EphA2's accumulation on the cell surface (30).

Overall, the increase in ligand-binding activity or a decrease in phosphatase expression that regulate the phosphotyrosine level may have caused the decrease in protein expression (26,30,31). Furthermore, ligand binding reverses the oncogenic effects of EphA2 overexpression (26).

This suggests the lack of correlation between EphA2 mRNA overexpression and clinicopathological data.

The overall results suggest that in HNSCC, the elevation of EphA2 mRNA may have initially occurred implicating its role in the early tumorigenesis of HNSCC, although the subsequent decrease in protein expression suggests that EphA2 may not be responsible for the progression of HNSCC.

In conclusion, EphA2 mRNA overexpression in HNSCC suggests EphA2 as a possible oncogene in its initial development. However, the lack of correlation between mRNA overexpression and clinicopathological data as well as the weak protein expression indicate that EphA2 does not play a major role in the progression of HNSCC. The specific involvement of EphA2 as well as the consideration of EphA2 as a target in the molecular therapy of HNSCC needs further evaluation.

## Acknowledgements

This study was partially supported by grants-in-aid for scientific research from the Ministry of Education, Culture, Sports, Science and Technology [19592109 (to HN), 18-06262 (to EG), 17406027 (to NN)], Seed Innovation Research from Japan Science and Technology Agency (to MG), from Sumitomo Trust Haraguchi Memorial Cancer Research Promotion (to MG) and AstraZeneca Research Grant (to MG). The authors would like to thank Ms. Masae Fujii and Ms. Kazuko Funakoshi for their technical expertise.

## References

1. Cheng N, Brantley DM and Chen J: The ephrins and Eph receptors in angiogenesis. Cytokine Growth Factor Rev 13: 75-85, 2002.
2. Durbin L, Brennan C, Shiomi K, *et al*: Eph signaling is required for segmentation and differentiation of the somites. Genes Dev 12: 3096-3109, 1998.
3. Friedman GC and O'Leary DD: Eph receptor tyrosine kinases and their ligands in neural development. Curr Opin Neurobiol 6: 127-133, 1996.
4. Lindberg RA and Hunter T: cDNA cloning and characterization of eck, an epithelial cell receptor protein-tyrosine kinase in the eph/elk family of protein kinases. Mol Cell Biol 10: 6316-6324, 1990.
5. Tuzi NL and Gullick WJ: eph, the largest known family of putative growth factor receptors. Br J Cancer 69: 417-421, 1994.
6. Rosenberg IM, Goke M, Kanai M, Reinecker HC and Podolsky DK: Epithelial cell kinase-B61: an autocrine loop modulating intestinal epithelial migration and barrier function. Am J Physiol 273: G824-G832, 1997.
7. Pandey A, Shao H, Marks RM, Polverini PJ and Dixit VM: Role of B61, the ligand for the Eck receptor tyrosine kinase, in TNF-alpha-induced angiogenesis. Science 268: 567-569, 1995.
8. Piaskowski S, Rieske P, Szybka M, *et al*: Atypical molecular background of glioblastoma and meningioma developed in a patient with Li-Fraumeni syndrome. J Neurooncol 71: 27-30, 2005.
9. Sulman EP, Tang XX, Allen C, Biegel JA, Pleasure DE, Brodeur GM and Ikegaki N: ECK, a human EPH-related gene, maps to 1p36.1, a common region of alteration in human cancers. Genomics 40: 371-374, 1997.
10. Griffin CA, Hruban RH, Long PP, Miller N, Volz P, Carson B and Brem H: Chromosome abnormalities in meningeal neoplasms: do they correlate with histology? Cancer Genet Cytogenet 78: 46-52, 1994.
11. White PS, Thompson PM, Gotoh T, *et al*: Definition and characterization of a region of 1p36.3 consistently deleted in neuroblastoma. Oncogene 24: 2684-2694, 2005.
12. Abraham S, Knapp DW, Cheng L, *et al*: Expression of EphA2 and Ephrin A-1 in carcinoma of the urinary bladder. Clin Cancer Res 12: 353-360, 2006.
13. Herrem CJ, Tatsumi T, Olson KS, *et al*: Expression of EphA2 is prognostic of disease-free interval and overall survival in surgically treated patients with renal cell carcinoma. Clin Cancer Res 11: 226-231, 2005.
14. Kinch MS, Moore MB and Harpole DH Jr: Predictive value of the EphA2 receptor tyrosine kinase in lung cancer recurrence and survival. Clin Cancer Res 9: 613-618, 2003.
15. Miyazaki T, Kato H, Fukuchi M, Nakajima M and Kuwano H: EphA2 overexpression correlates with poor prognosis in esophageal squamous cell carcinoma. Int J Cancer 103: 657-663, 2003.
16. Saito T, Masuda N, Miyazaki T, *et al*: Expression of EphA2 and E-cadherin in colorectal cancer: correlation with cancer metastasis. Oncol Rep 11: 605-611, 2004.
17. Thaker PH, Deavers M, Celestino J, *et al*: EphA2 expression is associated with aggressive features in ovarian carcinoma. Clin Cancer Res 10: 5145-5150, 2004.
18. Xu F, Zhong W, Li J, Shanshen Z, Cui J, Nesland JM and Suo Z: Predictive value of EphA2 and EphrinA-1 expression in oesophageal squamous cell carcinoma. Anticancer Res 25: 2943-2950, 2005.
19. Wu D, Suo Z, Kristensen GB, Li S, Troen G, Holm R and Nesland JM: Prognostic value of EphA2 and EphrinA-1 in squamous cell cervical carcinoma. Gynecol Oncol 94: 312-319, 2004.
20. Zelinski DP, Zantek ND, Stewart JC, Irizarry AR and Kinch MS: EphA2 overexpression causes tumorigenesis of mammary epithelial cells. Cancer Res 61: 2301-2306, 2001.
21. Gunduz M, Ouchida M, Fukushima K, *et al*: Genomic structure of the human ING1 gene and tumor-specific mutations detected in head and neck squamous cell carcinomas. Cancer Res 60: 3143-3146, 2000.
22. Gunduz M, Ouchida M, Fukushima K, *et al*: Allelic loss and reduced expression of the ING3, a candidate tumor suppressor gene at 7q31, in human head and neck cancers. Oncogene 21: 4462-4470, 2002.
23. Temnykh S, DeClerck G, Lukashova A, Lipovich L, Cartinhour S and McCouch S: Computational and experimental analysis of microsatellites in rice (*Oryza sativa L.*): frequency, length variation, transposon associations, and genetic marker potential. Genome Res 11: 1441-1452, 2001.
24. Parkin DM, Bray F, Ferlay J and Pisani P: Global cancer statistics, 2002. CA Cancer J Clin 55: 74-108, 2005.
25. Jemal A, Siegel R, Ward E, Murray T, Xu J, Smigal C and Thun MJ: Cancer statistics, 2006. CA Cancer J Clin 56: 106-130, 2006.
26. Walker-Daniels J, Riese DJ II and Kinch MS: c-Cbl-dependent EphA2 protein degradation is induced by ligand binding. Mol Cancer Res 1: 79-87, 2002.
27. Herath NI, Spanevello MD, Sabesan S, *et al*: Over-expression of Eph and ephrin genes in advanced ovarian cancer: ephrin gene expression correlates with shortened survival. BMC Cancer 6: 144, 2006.
28. Dohn M, Jiang J and Chen X: Receptor tyrosine kinase EphA2 is regulated by p53-family proteins and induces apoptosis. Oncogene 20: 6503-6515, 2001.
29. Zantek ND, Walker-Daniels J, Stewart J, *et al*: MCF-10A-NeoST: a new cell system for studying cell-ECM and cell-cell interactions in breast cancer. Clin Cancer Res 7: 3640-3648, 2001.
30. Kikawa KD, Vidale DR, Van Etten RL and Kinch MS: Regulation of the EphA2 kinase by the low molecular weight tyrosine phosphatase induces transformation. J Biol Chem 277: 39274-39279, 2002.
31. Parri M, Buricchi F, Taddei ML, Giannoni E, Raugei G, Ramponi G and Chiarugi P: EphrinA1 repulsive response is regulated by an EphA2 tyrosine phosphatase. J Biol Chem 280: 34008-34018, 2005.