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HER-2/neu Gene Amplification in Paraffin-Embedded Tissue Sections of Meningioma Patients

Meningiom Olgularına ait Parafin Blok Doku Kesitlerinde HER-2/neu Gen Amplifikasyonu

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ABSTRACT

AIM: Meningiomas arise from the meningoendothelial cells and are one of the most common tumors of the central nervous system. The HER-2/neu gene is located on the 17q11.2-q12 chromosome region and encodes an epidermal growth factor receptor. HER-2/neu gene amplification and/or over expression have been studied most widely in breast carcinomas. Previous studies have shown the importance of HER-2/neu gene amplification on the prognosis of meningioma cases. In this study, we aimed to detect HER-2/neu gene copy number in archive materials of 55 meningioma patients by fluorescent in situ hybridization (FISH).

MATERIAL and METHODS: The patients included in the study had undergone surgery in the neurosurgery department of our hospital between 1999 and 2002. Tissue samples were classified histologically according to WHO 2007 guidelines. Interphase FISH was performed on 3 to 4μm thick paraffin embedded tissue sections for the detection of HER-2/neu gene amplification status.

RESULTS: We found HER-2/neu gene amplification in 7 (12.73%) patients. Another 2 patients had only one signal for the HER-2/neu region. We confirmed this finding by a second hybridization with the chromosome 17p13.1 (p53) probe.

CONCLUSION: According to our results, HER-2/neu amplification could be regarded as an additional genetic factor playing role in meningioma pathogenesis together with known chromosomal abnormalities.

KEYWORDS: HER-2/neu, Gene Amplification, Meningioma

ÖZ

AMAÇ: Meningiomlar meningoendotelyal hücrelerden kaynaklanan, merkezi sinir sisteminin en sık tümörlerindendir. HER-2/neu geni 17. kromozomun q11.2-q12 bölgesinde yerleşik epidermal büyüme faktörü reseptörü kodlayan gendir. HER-2/neu gen amplifikasyonu ve/ veya fazla gen ifadelenmesi en sık meme karsinomlarında çalışılmıştır. Önceki çalışmalarda HER-2/neu gen amplifikasyonunun meningiom olgularının prognozundaki önemi vurgulanmıştır. Bu çalışmada 55 meningiom olgusunun arşiv materyalinde floresan in situ hibridizasyon (FISH) yöntemi ile HER-2/neu gen kopya sayısını belirlemeyi hedefledik.

YÖNTEM ve GEREÇ: Çalışma kapsamına 1999-2000 yılları arasında Beyin Cerrahisi Anabilim Dalında opere edilen olgular dahil edildi, Doku örnekleri histolojik olarak DSÖ 2007 kılavuzuna göre sınıflandırıldı. HER-2/neu gen amplifikasyonunun belirlenmesi amacıyla 3-4 μkalınlığındaki parafin blok doku kesitlerinde interfaz FISH yöntemi uygulandı.

BULGULAR: HER-2/neu gen amplifikasyonu 7 olguda (%12.73) gözlendi. Diğer iki olguda HER-2/neu bölgesi için tek sinyal gözlendi. Bu bulgu 17p13.1(p53) bölgesine özgü prob ile ikinci bir hibridizasyon ile doğrulandı.

SONUÇ: Bulgularımız ışığında HER-2/neu gen amplifikasyonunun meningiom patogenezinde bilinen kromozom anomalileri ile birlikte rol oynayan ek bir genetik faktör olarak değerlendirilebileceğini düşündük.

ANAHTAR SÖZCÜKLER: HER-2/neu, Gen amplifikasyonu, Meningiom

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INTRODUCTION

Meningiomas are common tumors of the central nervous system that arise from meningoendothelial cells of the spinal cord and brain. Meningiomas account for 30% of all primary brain tumors with an adjusted annual incidence of 2.3 per 100 000 individuals (1). These tumors have been most commonly reported in elderly patients with peak incidence in the seventh decade of life. There is a clear bias towards women with a female to male ratio of 2:1. Spinal meningiomas have an even greater predilection for females with a ratio of 10:1 (10).

Most meningiomas are slow-growing benign lesions and are typically associated with symptoms of gradually increasing intracranial pressure. Although the majority of these tumors are histologically benign, meningiomas have shown significant rates of recurrence, morbidity and mortality in studies with long-term follow-up (3). Clinical progress is difficult to predict. The World Health Organization (WHO) classifies meningiomas into three histological grades; grade I (benign), grade II (atypical), grade III (anaplastic) in accordance with the clinical prognosis. Recurrence is a complication that influences the patient's clinical course significantly (3).

Meningiomas are among the first solid human tumors in which consistent genetic aberrations have been demonstrated. Although the molecular alterations associated with progression to grade II and grade III are still poorly understood at present, the most common chromosome aberration in benign meningiomas is the loss of chromosome 22. Partial or complete loss of the short arm of chromosome 1 is the second most common aberration in meningioma and appears to characterize an early step in the progressive loss of chromosomal material in histologically atypical and anaplastic meningiomas (5).

HER-2/neu (also known as c-erB-2 or HER2) oncogene is the member of the epidermal growth factor receptor family. HER-2/neu encoded p185 protein is a receptor tyrosine kinase that can be associated with multiple signal transduction pathways. It has been found to be over-expressed in many different types of human malignancies, notably breast, ovarian, gastric, pancreatic, prostatic, colorectal, cancers of the female genital tract, and also recently, lung cancer (2,7,9).

In this study, we aimed to investigate HER-2/neu gene amplification by fluorescence in situ hybridization (FISH) in meningiomas to evaluate its prognostic value in the behavior of the disease.

PATIENTS and METHODS

Patients

Paraffin-embedded tissue sections of meningiomas from 55 patients who had undergone surgery at the Department of Neurosurgery between 1999 and 2002 were included in the study. The study was approved by the Institutional Clinical Research Ethics Committee. Of the patients, 43 were female and 12 were male with a mean age of 54.63. The histopathological diagnosis of meningioma had been established by standard light-microscopic evaluation of sections stained with hematoxylin and eosin in each case. The sections of each case were re-evaluated using the World Health Organization (WHO) 2007 criteria for brain tumor classification.

FISH Studies

Interphase FISH was performed on 3 to 4 μm-thick paraffin embedded tissue sections obtained from all patients for the detection of HER2 gene amplification. The tissue sections were placed on poly-L-Lysine coated slides and deparaffinized with a procedure that was slightly modified than previously described. After deparaffinization at 56°C overnight, xylene dehydration in alcohol, pepsin digestion and fixation of slides, denaturation and hybridization were carried out according to the manufacturer's information for each probe in the HyBrite denaturation/ hybridization system for FISH (Vysis Inc., IL). Slides were denatured in 70% formamide, 2 x SSC at 75°C for 3-5 min and the probe [Chromosome 17q12 (HER-2/neu)/ alphasatellite 17 cocktail, dual color, direct labeled (Oncor, Qbiogene, UK)] was denatured at 96°C for 5 min. Hybridization was carried out at 37°C for 14-16 h. The slides were then washed in posthybridization wash buffer at 65°C for 5 min and counterstained with DAPI.

Signals were counted in at least 200 cells for both the HER-2/neu gene and chromosome 17 centromere signals under oil immersion at x1000 magnification using the recommended filters. Results were expressed as the ratio of HER-2/neu signal (orange) to centromere 17 signal (green). The expected ratio is 1; which means that there is no gene amplification and a ratio of signals ≥2 is regarded as Her2 /neu gene amplification.

RESULTS

Paraffin block sections from 55 patients with different types of sporadic meningiomas were included in our study. According to the WHO classification, 83.65% of the tumors were classified as Grade I, 14.54% as Grade II (or atypical), and 1.81% as Grade III (or anaplastic) meningiomas.

We found increased HER-2/neu gene copy number in 7 patients (Figure 1). One patient was classified as grade 2 meningioma and another was Grade 3. Two patients had only one signal for the HER-2/neu region (Figure 2). We confirmed this finding by a second hybridization with the chromosome 17p13.1 (p53) (Vysis, UK) probe. The results and clinical features of the patients with FISH findings are shown in the Table and Figure 2.

DISCUSSION

The potential role of HER-2/neu amplification status is an indicator of choice for targeted therapeutic regimens in cancer patients. In a previous study, we showed that HER-2/neu status should be determined in breast cancer patients when planning therapy regimens and during follow-up (7).

There are only a few studies of HER-2/neu expression in meningiomas. In one study, HER-2/neu expression levels were evaluated with different methods and it was concluded that the over-expression of HER-2/neu protein is associated in some meningiomas with an increased number of HER-2/neu gene copies (1).

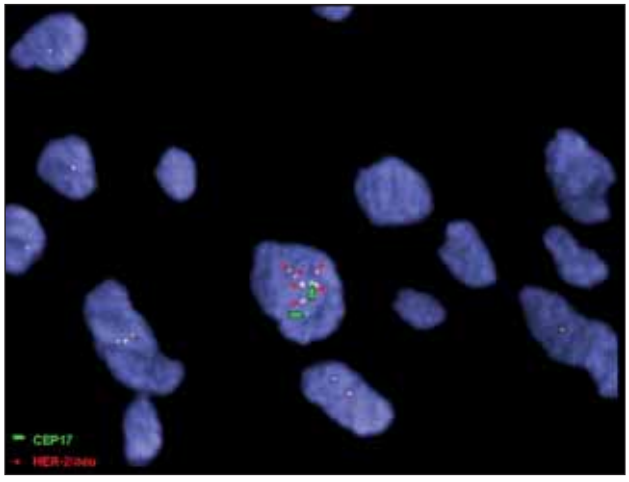

Figure 1: *FISH results of HER-2/neu gene amplification in patient number 2. Orange signals correspond to HER-2/neu gene and green signals correspond to chromosome 17 centromere.*

Table I: Demographic characteristics of Her2/neu gene amplification detected patients and their tumor grades

Patient No	Age	Sex	Histopathological Grade	Signal Ratio
1	50	M	Grade III	2
2	38	F	Grade I	3
3	36	M	Grade I	2
4	72	M	Grade I	2
5	60	F	Grade II	4
6	41	M	Grade I	2
7	51	F	Grade I	2

M: Male, F: Female

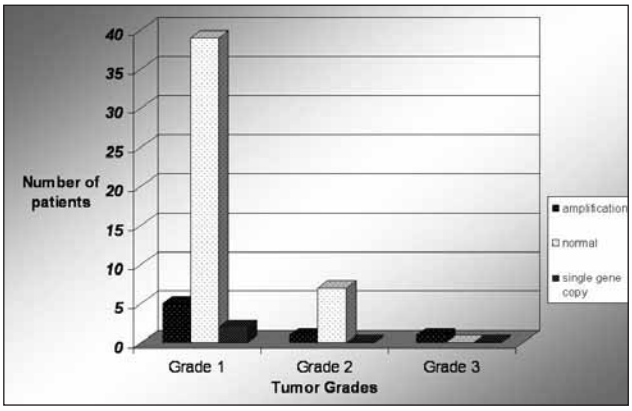

Figure 2: *Amplification status and tumor grades of the patients included in the study.*

About 80% of all meningiomas are slow-growing tumors of WHO grade I. The basic genetic mechanism underlying the development of benign meningiomas is alterations on the long arm of chromosome 22. We have previously shown that 22q deletions were found 72.2% of Grade I meningioma patients but suggested that there was no correlation of tumor recurrence with 22q deletions (11).

In this study, we found HER-2/neu gene amplification in 5 patients with Grade I, one patient with Grade 2 and one patient with Grade 3 meningiomas and we suggest that HER-2/neu gene amplification might have an impact on the different stages of oncogenesis of this tumor.

Previously, the rate of recurrence was found to be significantly higher in HER-2/neu positive meningiomas than in HER-2/neu negative

meningiomas. It is discussed that HER-2/neu might be an independent prognostic factor in foreseeing the recurrence of meningiomas (4). However, there was no HER-2/neu gene amplification in 2 patients with tumor recurrence in our study.

Atypical (Grade II) and anaplastic (Grade III) meningiomas constitute 15–20% and 1-3% of meningiomas, respectively. Complex genetic changes and molecular pathways might affect the progression from benign to atypical and anaplastic meningioma (5). These complex changes, when occurring in genes like TP53, NF1, RPS6KB1 which are situated on different parts of chromosome 17 and responsible for cell cycle control and growth, are thought to be associated with a worse prognosis in meningioma. Alterations such as gains, deletions and amplifications in these genes might be important in the malignant transformation of Grade 1 meningiomas (6,8). In our study we found HER-2/neu gene amplification in one patient with Grade III meningioma, but our group is too small to associate results with disease progression.

In summary, although we found HER-2/neu gene amplification in meningioma patients, it is not easy to reach a conclusion that HER-2/neu amplification has an impact on disease clinical course in meningiomas due to the limited number of cases studied. Given the genetic heterogeneity of this disease, the investigation of HER-2/neu amplification along with frequent genetic changes would be valuable in enlightening the tumor biology and determining the prognostic factors.

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