Original Article / Çalışma - Araştırma

Investigating KRAS/BRAF mutation in oropharyngeal squamous cell carcinomas: a preliminary study

Orofarengeal skuamöz hücreli karsinomlarda KRAS/BRAF mutasyonunun araştırılması: Bir ön çalışma

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ABSTRACT

Objectives: This study aims to investigate the role of KRAS/BRAF gene mutation in the pathogenesis of oropharyngeal squamous cell carcinoma (OSCC).

Patients and Methods: A total of 26 OSCC patients (23 males, 3 females; mean age 60 years; range 41 to 77 years) diagnosed between January 2003 and November 2013 were included in the study. The methods used in our study were quantitative fluorescence polymerase chain reaction for KRAS/BRAF mutation analysis.

Results: Ten of the tumors were located at the tongue base, 12 in the tonsil and four at the floor of mouth. The mean tumor size was 3.8 cm. Six of the tumors were well differentiated, 18 were moderately differentiated and two were poorly differentiated. All cases were analyzed for KRAS and BRAF gene mutations and none of them showed gene mutations.

Conclusion: We could not find any relation between OSCC and KRAS/BRAF gene mutations in our short case file. The role of mutations should be analyzed in larger series in OSCC to predict new targeted therapy modalities.

Keywords: BRAF; KRAS; oropharyngeal; squamous cell carcinoma.

ÖZ

Amaç: Bu çalışmada orofarengeal skuamöz hücreli karsinom (OSCC) patogenezinde KRAS/BRAF gen mutasyonunun rolü araştırıldı.

Hastalar ve Yöntemler: Ocak 2003-Kasım 2013 tarihleri arasında tanısı konan toplam 26 OSCC hastası (23 erkek, 3 kadın; ort. yaş 60 yıl; dağılım 41-77 yıl) çalışmaya dahil edildi. Çalışmada KRAS/BRAF mutasyon analizi için kantitatif floresan polimeraz zincir reaksiyonu yöntemi kullanıldı.

Bulgular: Tümörlerin 10'u dil kökünde, 12'si bademcikte ve dördü ağız tabanı yerleşimli idi. Ortalama tümör boyutu 3.8 cm idi. Tümörlerin altısı iyi diferansiye, 18'si orta derecede diferansiye ve ikisi kötü diferansiye idi. Tüm olgular KRAS ve BRAF gen mutasyonları için analiz edildi ve bunların hiçbirinde mutasyon saptanmadı.

Sonuç: Sınırlı sayıdaki olgu serimizde, OSCC ile KRAS/BRAF gen mutasyonu arasında herhangi bir ilişki saptanamamıştır. Yeni hedefe yönelik tedavi yöntemlerini belirleyebilmek için daha geniş OSCC serilerinde mutasyonun rolü araştırılmalıdır.

Anahtar Sözcükler: BRAF; KRAS; orofarengeal; skuamöz hücreli karsinom.

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Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer over the worldwide.[1] Survival rates are still low in spite of the efforts to improve conventional treatment.[2]

Determining the underlying factors in cancer etiology may shed light on the development of targeted therapy modalities.[2] Hence, molecular biology has become one of the most popular research areas in recent years.

RAS is one of the major oncogenes in cancer pathogenesis. KRAS gene mutation is detected in 15-30% of solid tumors.[2] Though RAS genes are rarely mutated in HNSCC, there are some studies reporting mutation of the RAS family.[3,4]

Proto-oncogene serine/threonine protein kinase (BRAF) mutation is observed in 15% of human malignancies especially in malignant melanomas[5] although the role of BRAF mutation in HNSCC is controversial. Our aim is to investigate the role of KRAS/BRAF gene mutation in the pathogenesis of oropharyngeal squamous cell carcinoma (OSCC).

PATIENTS AND METHODS

A total of 26 OSCC patients (23 males, 3 females; mean age 60 years; range 41 to 77 years) diagnosed between January 2003 and November 2013 were included in this study. Data about age, gender and tumor site (tongue base, floor of mouth and tonsil) were obtained from medical files. Tumor diameters were obtained from radiology and pathology reports. Hematoxylin-eosin stained tumor sections were re-evaluated and the best representative tumor blocks were chosen. The methods used in our study were quantitative flourescent-polymerase chain reaction (QF-PCR) for KRAS/BRAF mutation analysis. The study protocol was approved by the Tepecik Training and Research Hospital Ethic Committee. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Mutation analysis

DNA was extracted from formalin-fixed, paraffin-embedded tissues (FFPE) sections using the QIAamp FFPE Tissue kit (Qiagen Inc., Valencia, CA, USA) according to the manufacturer's instructions, with the addition of a 16-hour proteinase K lysis step. QF-PCR was carried out with a commercially available KRAS and BRAF mutation screening kit (Devyser, Hägersten, Sweeden). After preanalytic amplification control (PAC) for confirmation of presence of amplifiable DNA, a single tube, multiplex, PCR assay was performed. Capillary electrophoresis was used for detection of mutation specific PCR fragments with ABI3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

RESULTS

Of the 26 samples, 10 tumors (38.5%) were located at the tongue base, 12 (46.1%) in the tonsil and 4 (15.4%) in the floor of mouth. The mean tumor size was 3.8 cm (min: 1 cm, max: 8 cm). The tumors were classified as well/moderately/poorly differentiated according to their histological differentiation. Six tumors (23.1%) were well differentiated, 18 (69.2%) were moderately differentiated and two (7.7%) were poorly differentiated. All cases were analyzed for KRAS/BRAF gene mutations and none of them showed gene mutations.

DISCUSSION

Overall survival (OS) rate of HNSCC is reported to be low despite of the devoloping surgical techniques, new chemotherapeutic agents, combined use of chemotherapy and radiotherapy.[6] The five year OS rate is still around 50%.[6] Molecular studies are ongoing to understand HNSCC carcinogenesis. These novel findings can shed light on targeted therapy modalities.[7]

KRAS gene mutation prevalence in human solid tumors is 15-30%, and 40% in colorectal cancer.[2,8] The role of KRAS mutation in HNSCC is controversial. Smilek et al.[9] examined the KRAS mutation status of 27 HNSCC patients and found alterations in 14.8% (4/27) of the cases. Weber et al.[10] found a KRAS mutation in 6% (3/89) of HNSCC samples while Van Damme et al.[11] identified mutations in 4.5% (1/22) of the HNSCC specimens. Bruckman et al.[12] found a KRAS mutation in 2.4% (1/42) of OSCC samples.

Bissada et al.[2] found a KRAS mutation in only seven (4 oropharynx, 3 larynx) of 195 HNSCC cases. On the other hand Friedland et al.[13] found a KRAS mutation in none of 60 HNSCC cases. Fujii et al.[14] did not detect a KRAS mutation among 183 HNSCC samples.

BRAF mutation has been identified in approximately 15% of all human cancers especially in malignant melanomas.[5] A BRAF mutation was reported in 3% of 89 cases by Weber et al.,[10] and 1.6% of 60 cases by Friedland et al.[13] in a HNSCC case series. A study evaluating HNSCC patients reported BRAF mutation as 2.4% (1/42).[12] De Carvalho et al.[7] found no mutation in 94 HNSCC cases. In our series none of the 26 OSCC cases showed KRAS/BRAF gene mutations consistent with the large HNSCC literature data.

KRAS/BRAF pathways can be activated by other mechanisms than gene mutation. According to Friedland et al.[13] despite low KRAS/BRAF mutation rates in HNSCC, activation of these genes by other mechanisms may still play a role in HNSCC development.

KRAS mutation status which is the major predictor of response to cetuximab therapy has been well documented in colorectal cancer.[14] However KRAS mutation is rare in OSCC, and investigating the KRAS mutation status in all OSCC patients remains controversial.

In conclusion, the survival rates of OSCC vary between individual cases according to histopathological factors and molecular alterations. We tried to find such molecular changes that can be target of therapies and also predictors of prognosis. We could not find any relation between OSCC and KRAS/BRAF gene mutation in our study. But predicting KRAS mutation can be essential in cases that are candidates for cetuximab treatment. The role of mutations should be analyzed in larger clinical trials in OSCC to predict new targeted therapy modalities and prognosis.

Declaration of conflicting interests

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