Short Communication

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Research Journal of Oncology

2018
Vol.2 No.1:4

EGFR Gene Status Correlates with Erk Pathway Activation and Poor Outcome in HPV-Negative Oropharyngeal and Sinonasal Carcinomas

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Abstract

Background: Personalized medicine requires a deep understanding of the molecular profile of tumours. Our aim was to investigate major biomarkers in head and neck carcinomas, including HPV-infection, EGFR gene and the ERK pathway activation.

Methods and findings: Carcinomas from the oropharynx (n=55) and sinonasal tract (n=51) were included. HPV was analyzed through p16 immunostain and PCR; EGFR gene expression and copy number, by immunohistochemistry and FISH, respectively. The activation of the ERK pathway was investigated through the expression of ERK1/2.

Inverse relationship between HPV-infection and EGFR gene copy number was found ($p<0.01$). EGFR gene copy number and expression correlated with ERK1/2 expression ($p<0.001$). Overall survival correlated directly with HPV infection ($p=0.004$) and inversely with EGFR copy number ($p=0.016$) and ERK1/2 expression ($p=0.015$).

Conclusions: High-EGFR gene copy number and expression usually involve HPV-negative carcinomas and correlate with ERK1/2 pathway activation and poor outcome. These results may have a high translational relevance.

Keywords: HPV; EGFR; p16; ERK; Sinonasal carcinoma; Oropharyngeal carcinoma

Received: May 14, 2018; **Accepted:** May 23, 2018; **Published:** June 01, 2018

Citation: Castillo P, Diaz A, Hakim S, Larque AB, Ordi J, et al. (2018) EGFR Gene Status Correlates with Erk Pathway Activation and Poor Outcome in HPV-Negative Oropharyngeal and Sinonasal Carcinomas. Res J Oncol Vol.2 No.1:4

Introduction

The incidence of head and neck squamous cell carcinoma (HNSCC) is estimated worldwide in 700,000 cases annually [1]. Most HNSCC are diagnosed in advanced stages of the disease, leading to high morbidity and mortality rates. Consequently, there is an increasing need to expand the molecular knowledge of the complex process of malignant transformation in order to identify the prognosis and predictive markers, as well as new therapeutic approaches.

In the last few decades, remarkable advances have been made in the characterization of HNSCC. Human papilloma virus (HPV) is recognized as an increasing cause of a subtype of HNSCC 2, more frequently found in the oropharynx [2] and the sinonasal tract [3,4]. The relevance in detecting the HPV- associated HNSCC is due to the different responses to radiotherapy and chemotherapy of these tumours, resulting in a significantly better prognosis for the patients [5].

Additionally, the epidermal growth factor receptor (EGFR) has emerged as a significant factor in the development and growth of HNSCC. Major signalling route of EGFR is the RAS-RAF-Mitogen activated protein kinase (MAPK) pathway which leads to the activation of MAPKs [6]. The MAPKs extracellular signal-regulated kinases ERK1/2 are the best characterized and most strongly associated with human cancer. The activation of this pathway promotes cell proliferation, invasion, and metastasis.

The aim of this study is to establish the impact of major biomarkers, including HPV-infection, EGFR gene status and ERK pathway activation, in a series of oropharyngeal and sinonasal carcinomas, in order to contribute to the understanding of

prognostic factors and the development of tailored treatment approaches.

Materials and Methods

Study settings and design

This retrospective study was conducted at the Department of Pathology of the Hospital Clinic, University of Barcelona and received the approval of the Clinical Research Ethics Committee of the Hospital Clinic of Barcelona. The cohort included 106 patients with HNSCC: 55 (52%) from the oropharynx and 51 (48%) from the sinonasal tract region. All patients were followed-up in the Oncology Department of the same hospital.

DNA extraction and HPV detection and typing

Detailed description of the DNA extraction, HPV detection and typing is reported elsewhere 3. A broad-spectrum HPV DNA amplification was performed using the short PCR fragment (SPF10) primer set (Innogenetics Diagnostica, Spain). SPF10 amplimers from DEIA-HPV positive samples were analyzed by reverse hybridization in an HPV line-probe assay LiPA25 system version 1 (Labo Bio-medical Products, Rijswijk, The Netherlands) at high stringency, generating a type-specific hybridization pattern. The HPV LiPA25 version 1 permits specific detection of 25 HPV types: HPV 6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68 or 73, 70 and 74.

Immunohistochemistry

Immunohistochemical studies for p16INK4A, EGFR and ERK1/2 were performed with the automated immunohistochemical system TechMate 500® (Dako Co, Carpinteria, CA), using the EnVision system (Dako) following previously reported protocols [7].

Fluorescent in situ Hybridization (FISH) for EGFR gene: FISH was performed following standard protocols previously reported 7 using the dual-colour EGFR Spectrum-red/CEP7 Spectrum-blue probe (Dako).

Statistical analysis

Data were analyzed with the program SPSS version 23.0 (SPSS Inc, Chicago, IL, USA). Chi-square analysis or Fisher's exact test were used for comparisons between qualitative variables, and Student's t test and ANOVA were applied for quantitative variables according to application conditions. Progression free and overall survivals were analyzed by the Kaplan-Meier method. Differences were analyzed by the Log-Rank method. Differences were considered of statistical significance with α risk of 0.05.

Results

Table 1 shows the clinicopathological characteristics, molecular and immunohistochemical results of the patients according to the HPV-status.

Characteristics of the patients and HPV detection

HPV16 was detected in 25 cases (24% of all tumours), most of

Table 1 Clinicopathological characteristics of the patients, molecular and immunohistochemical results in Human Papillomavirus–Positive and Negative Tumours.

	HPV positive; n= 25	(%)	HPV negative; n= 81	(%)	*p*
Age (years)					
Median (range)	64		61		0.404
Gender					**0.007**
Female	10	-40	12	-15	
Male	15	-60	69	-85	
Smoking history					**0.013**
Yes	16	-64	65	-80	
No	7	-28	10	-12	
Unknown	2	-8	6	-7	
Site of tumour					
Oropharynx	15	-60	40	-49	0.353
Sinonasal tract	10	-40	41	-51	
Stage					0.502
I	1	-4	5	-6	
II	5	-20	18	-22	
III	4	-16	22	-27	
IV	14	-56	32	-40	
Not available	1	-4	2	-2	
p16 -IHC[1]					**<0.001**
Positive	24	-96	4	-5	
Negative	1	-4	77	-95	
EGFR-FISH[2]					**<0.001**
Positive	1	-4	32	-78	
Negative	24	-96	46	-19	
EGFR-IHC [3]					0.079
Positive	15	-60	66	-81	
Negative	10	-40	14	-17	
ERK1/2- IHC[4]					**<0.001**
Negative	7	-28	11	-14	
Positive/low expression	11	-44	11	-14	
Positive/high expression	6	-24	54	-67	

Notes:

(1) Immunohistochemistry for p16. p16 positivity was considered when strong, diffuse nuclear and cytoplasmic staining of tumoural cells was shown.

(2) Three cases were not suitable for EGFR-FISH. High-polysomy was considered when ≥ 4 blue and red signals (in equal number) were seen in each nucleus. EGFR amplification was defined as red signals > 2 blue signals. High-polysomy and amplification were considered positive.

(3) Immunohistochemistry for EGFR. One case was not valid for evaluation. The immunostaining for EGFR was quantitatively scored in 3 grades, according to staining intensity and extension (Lujan 2010). Only cases with 2+ and 3+ staining patterns were considered positive.

(4) Immunohistochemistry for ERK1/2. Six cases were not available for analysis. Positivity for pERK1/2 was considered when nuclear staining ≥ 10% tumoural cells; and high-pERK1/2 expression, when nuclear staining ≥ 30% tumoural cells.

which were located in the oropharynx (15/25, 60%). Following the initial biopsy, the patients were treated by surgery and radiotherapy or chemoradiotherapy, following the Oncology Department protocols.

EGFR gene copy number, EGFR and ERK1/2 expression

EGFR gene alterations included 28 high-polysomies (85%) and 5 amplifications (15%). High-pERK1/2 expression was identified mostly in HPV negative patients ($p<0.001$), and correlated with the EGFR gene copy number ($p<0.001$). EGFR gene high-polysomy and amplification was associated with EGFR protein overexpression ($p=0.011$) and high-ERK1/2 expression ($p=0.002$).

No statistical correlation was identified between EGFR copy gain and the location of tumours (oropharynx vs. sinonasal), age, gender or stage of tumours.

Survival analysis in relation to the HPV and EGFR status

HPV positive patients had a longer progression free survival ($p=0.002$) and overall survival ($p=0.004$). Patients with high-EGFR gene copy number and patients with high-ERK1/2 expression showed worse overall survival ($p=0.016$ and $p=0.015$ respectively).

Discussion

This study shows that carcinomas from the oropharynx and sinonasal tract with high- EGFR gene copy number are usually HPV-negative and harbour the ERK1/2 pathway activation. In addition, we have found a significantly better outcome of HPV-associated carcinomas, whereas the tumours with high-EGFR copy number and ERK1/2 pathway activation are associated with a worse outcome.

In this study EGFR gene gain was seen in 32% of the cases, a higher number than those observed in other studies on oropharyngeal carcinomas [8]. This discrepancy can be explained by the high number of smoker patients with HPV- negative tumours in our cohort. Additionally, the inclusion of sinonasal carcinomas with a lower rate of HPV-association could contribute to this difference.

The HPV involved in HNSCC carcinogenesis are high-risk genital type HPV, especially HPV16 type, according to our findings. HPV-positive tumours differ from those of HPV-negative in terms of risk factors, immunohistochemistry, molecular features, and response to radio and chemotherapy, indicating that these HNSCCs represent a separate tumour entity. Our results are consistent with the findings in previous reports on the genomics of oropharyngeal carcinomas which state that EGFR alterations largely exclude HPV-positive tumours [8]. However, this is the first study in which the inverse relationship between HPV-infection and high-EGFR gene copies is also shown in sinonasal carcinomas. These results likely extend to all HNSCCs, regardless of their location in the upper respiratory tract. We confirm that HPV infection and EGFR gene status define two molecularly different HNSCC subtypes, and the MAPK pathway activation is mainly shown in the HPV-negative tumours with high-EGFR copy number and expression. In fact, MAPK pathway activation through phosphorylation and expression of ERK1/2 is a relevant downstream component of EGFR signalling. According to our results, HNSCCs harbouring high-EGFR gene copies have been associated with a worse outcome in previously published studies [7,8]. In addition, ERK1/2 expression has also been associated with an aggressive tumoral behaviour in other tumour-types 7.

These results may have a high translational relevance. In recent years, EGFR has become a target for therapy, and EGFR-antagonists are included in the treatment protocols of HNSCC in advanced stages [9]. Nevertheless, previous studies on cell lines from HNSCCs have shown that activated ERK1/2 pathway provide protection against radiation-induced cell death. This radioresistance can be relieved through the effect of signaling pathway inhibitors [10].

Conclusion

HPV-infection and EGFR gene status classify HNSCCs into molecular groups with prognostic significance and high translational relevance. This study supports the implementation of new therapeutic strategies in HNSCCs, as the de-escalated treatment for HPV-associated tumours and the targeted adjuvant therapy with EGFR antagonists or multikinase inhibitors for carcinomas with high-EGFR gene copies.

Financial Disclosures

There are no financial disclosures from any authors.

Grants: This work was partly funded by Instituto de Salud Carlos III (ICSiii)-Fondos de Investigacion Sanitaria and ERDF 'one way to Europe' (PI11/01570) and the Fundacion Mutua Madrilena (FMM 2011 AP94722011).

Conflicts of Interest

None declared.

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