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Overexpression of *PIK3CA* in head and neck squamous cell carcinoma is associated with poor outcome and activation of the YAP pathway

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ABSTRACT

Objectives: Phosphatidylinositol 3-kinase catalytic subunit alpha (*PIK3CA*) is commonly altered in many human tumors, leading to the activation of p110α enzymatic activity that stimulates growth factor-independent cell growth. *PIK3CA* alterations such as mutation, gene amplification and overexpression are common in head and neck squamous cell carcinoma (HNSCC) and. We aim to explore how these alterations and clinical outcome are associated, as well as the molecular mechanisms involved.

Material and methods: Mutation and copy-number variation in *PIK3CA*, and whole-genome expression profiles, were analyzed in primary HNSCC tumors from The Cancer Genome Atlas (TCGA) cohort (n = 243). The results were validated in an independent cohort form the University Hospital of A Coruña (UHAC, n = 62). Expression of the *PIK3CA* gene protein product (PI3K p110α) and nuclear YAP were assessed in tissue microarrays in a cohort from the University Hospital 12 de Octubre (UH12O, n = 91).

Results: Only high expression of the *PIK3CA* gene was associated with poor clinical outcome. The study of gene expression, transcription factor and protein signatures suggested that the activation of the Hippo-YAP pathway, involved in organ size, stem cell maintenance and tumorigenesis, could underlie tumor progression in *PI3KCA* overexpressing tumors. Tissue arrays showed that PI3K p110α levels correlated with YAP nuclear localization in HNSCC tumors.

Conclusions: High expression of *PIK3CA* in HNSCC primary tumors identifies patients at high risk for recurrence. In these tumors, progression could rely on the Hippo-YAP pathway instead of the canonical Akt/mTOR pathway. This observation could have important implications in the therapeutic options for patients.

Introduction

Head and neck squamous cell carcinoma (HNSCC) is the sixth most prevalent type of cancer worldwide and eighth most common by cause of death. Each year nearly 700,000 people are diagnosed with HNSCC and more than half of them will die within 5 years [1,2]. This cancer arises in the upper aerodigestive tract, comprising the nasal cavity and paranasal sinuses, oral cavity, pharynx, larynx and trachea. The main risk factors associated with its development are tobacco and alcohol consumption, which have a synergistic effect when combined, and human papillomavirus (HPV) infection. HPV-positive tumors represent a subgroup with distinct tumor biology, clinical presentation and

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clinical outcome [3]. In fact, the presence of high-risk HPV in oropharyngeal cancer is the only molecular marker routinely used for risk stratification.

Current clinical assessment of HNSCC patients includes cervical lymph node metastasis [4]. This is a frequent event in this pathology and is a major determinant of poor prognosis and treatment design. However, depending on the techniques used, nodal metastases remain undetected during diagnostic workup in 20–40% of all patients [5–7]. We need more reliable markers that can be used to divide patients into distinct subclasses with different outcome rates and therapeutic options. The study of cancer genome has proven to be a valuable tool for diagnosis, classification and prognosis. In this sense, studies of the genomic alterations in HNSCC [8–17], including point-mutations, genome-wide copy number alterations (CNA) and gene expression, are contributing to the identification of biomarkers capable of predicting poor outcome or candidates for specific molecularly targeted anticancer agents. Despite these advances, no biomarker-based patient selection is currently being used for the treatment with the two molecularly-based anticancer therapies approved for HNSCC, namely Cetuximab and Pembrolizumab [18,19].

The *PIK3CA* oncogene encodes for the catalytic subunit alpha (p110α) of class IA PI3K (phosphoinositide 3-kinase) and is normally involved in the regulation of cell survival, growth and metabolism. It is frequently activated in human cancers [20] resulting in cellular growth advantage, evasion of apoptosis and invasion, thus contributing to tumor formation and progression. Genomic alterations in *PIK3CA* are common in HNSCC [9,11–15], affecting about 55% of cases, and include activating mutation, amplification and/or overexpression. PI3K signaling pathway is complex, with several downstream mediators, among which Akt kinase is considered central [21]. This pathway is an attractive therapeutic target in HNSCC since there are several inhibitors of PI3K and other mediators of the pathway in clinical use or undergoing clinical trials [22,23].

In this study, we evaluated the alteration in the *PIK3CA* gene as a prognostic marker in HNSCC. For this purpose we analyzed The Cancer Genome Atlas (TCGA) HPV-negative HNSCC cohort, the largest currently available with genomic and clinical data [14]. We validated our results in an independent cohort from the University Hospital of A Coruña (UHAC). The results revealed that *PIK3CA* overexpression, but not mutation, is a poor prognostic marker in head and neck squamous cell carcinoma. Our data suggest that the molecular pathways downstream of PI3K involved in HNSCC tumor progression triggered by *PIK3CA* mutation or overexpression might be different. This could have important implications when treating these patients with PI3K pathway inhibitors.

Materials and methods

Patients and samples for genomic analyses

Sixty-two primary cancer tissue samples were obtained from newly diagnosed patients at the time of tumor resection at the University Hospital of A Coruña (UHAC), A Coruña, Spain. Written consent from all patients and institutional review board approval from the hospital were obtained. Tumors were histologically diagnosed as HNSCC, and staged and graded according to the American Joint Committee on Cancer. Tumors selected for this study were HPV16 negative. Tumor samples were stored in TRIzol® (Thermo Fisher, Waltham, MA) following manufacturer's instructions at UHAC and sent to CIEMAT for molecular analysis.

Pathologic characteristics of the tumors and clinical follow-up data of the patients are summarized in Table 1. Further characteristics of the patients, tumors and outcome from the UHAC cohort appear in Supplementary Table S1. Full data from the TCGA cohort can be accessed via the original publication [14] or the cBioportal (http://www.cbioportal.org/) web tool. The full TCGA HPV negative cohort (n = 243) was employed for the analyses involving *PIK3CA* gene status and mRNA expression data (*PIK3CA* mutation, CNV and expression levels; molecular classification of mRNA subtypes; gene ontology; gene set enrichment analysis and transcription factor analysis). For analyses involving disease status, only the tumors belonging to patients with disease status data (n = 159) were analyzed.

Table 1
Characteristics of patients in the TCGA and UHAC Cohorts.

Variables	TCGA discovery cohort All *N* = 243 No. (%)*	UHAC validation cohort All *N* = 62 No. (%)*	*P*†
Age at diagnosis, yr			0.005
Median	62	68	
Range	19–90	33–90	
Sex			0.02
Male	171 (70)	34 (55)	
Female	72 (30)	28 (45)	
Smoker	n = 236		< 0.001
Yes[a]	194 (82)	38 (61)	
No	42 (18)	24 (39)	
Pathologic T status[b]	n = 240		0.02
T1-3	133 (55)	48 (77)	
T4	87 (36)	14 (23)	
TX	20 (8)		
Pathologic N status[c]	n = 239		< 0.001
Negative	80 (33)	44 (71)	
Positive	115 (48)	18 (29)	
NX	44 (18)		
OS, months			NA
Median	34.1	ND	
Range	18.1–50.0	ND	
Media	70.9	42.5	
Range	56.8–85.0	36.9–48.2	
Disease status	n = 159		0.55
Disease free	97 (61)	35 (56)	
Recurred/Progressed	62 (39)	27 (44)	
TTR, months			NA
Median	107.8	ND	
Range	20.7–194.9	ND	
Media	94.9	38.0	
Range	77.9–111.9	31.9–44.2	
PIK3CA gene mutation			1.0
Wild type	198 (81)	51 (82)	
Mutation	45 (19)	11 (18)	
PIK3CA gene copy number		n = 59	0.11
No amplification	194 (80)	41 (69)	
Amplification	49 (20)	18 (31)	
PIK3CA mRNA expression		n = 31	0.40
Low expression	175 (72)	20 (65)	
High expression	68 (28)	11 (35)	

Abbreviations: TX, primary tumor cannot be assessed; NX, regional lymph nodes could not be assessed; OS, overall survival; TTR, time to recurrence; NA, not applicable, months clinical follow-up data is different in the two cohorts; ND, not defined.

* Percentages may not total 100 because of rounding.

† *P* values were obtained by Mann-Whitney test for continuous variables and two-sided Fisher's exact test for categorical variables.

[a] Refers to "ever a smoker".

[b] Refers to primary tumor pathologic spread.

[c] Lymph node pathologic spread.

Determination of PIK3CA gene copy number and mutation

Genomic DNA was purified using DNeasy Blood and Tissue (Qiagen, Gaithersburg, MD) according to the manufacturer's instructions. DNA yield and purity were verified spectrophotometrically. *PIK3CA* gene