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Original Research

Does smoking alter the mutation profile of human papillomavirus–driven head and neck cancers?

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Abstract ***Background:*** Human papillomavirus (HPV)-driven oropharyngeal cancer (OPC) patients are characterised by a better prognosis than their HPV-negative counterparts. However, this significant survival advantage is not homogeneous and among HPV-positive patients those with a smoking history have a significantly increased risk of oncologic failure. The reason why tobacco consumption impacts negatively the prognosis is still elusive. Tobacco might induce additional genetic alterations leading to a more aggressive phenotype. The purpose of this study was to characterise the mutational profile of HPV-positive OPCs by smoking status. We hypothesise a higher frequency of mutations affecting smokers.
Methods: Targeted next-generation sequencing of 39 genes that are recurrently mutated in head and neck cancers (HNCs) caused by tobacco/alcohol consumption was performed in 62 HPV-driven OPC cases including smokers and non-smokers.
Results: The study population included 37 (60%) non-smokers and 25 (40%) smokers. Twenty (32%) patients had no mutation, 14 (23%) had 1 mutation and 28 (45%) had 2 or more mutations. The most commonly mutated genes regardless of tobacco consumption were PIK3CA (19%), MLL2 (19%), TP53 (8%), FAT 1 (15%), FBXW7 (16%), NOTCH1 (10%) and FGFR3 (10%). Mutation rate was not significantly different in smokers compared with non-smokers

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even when analyses focused on heavy smokers (>20 pack-years vs. <20 pack-years). Similarly, there was no significant difference in mutations patterns according to tobacco consumption. ***Conclusion:*** In HPV-positive patients, smoking does not increase the mutation rate of genes that are recurrently mutated in traditional HNC. Additional studies are warranted to further describe the molecular landscape of HPV-driven OPC according to tobacco consumption.

1. Introduction

Human papillomavirus (HPV)-driven oropharyngeal cancer (OPC) patients are characterised by a better prognosis than their HPV-negative counterparts with a mortality risk reduction ranging from 28 to 58% [1,2]. However, this significant survival advantage is not homogeneous, and many studies have highlighted that among HPV-positive patients those with a smoking history have a significantly increased risk of disease progression and death compared with those who have never smoked [3–8]. The reason why tobacco consumption impacts negatively the prognosis is still elusive. Tobacco might induce additional genetic alterations leading to a more aggressive phenotype and an increased likelihood of resistance to therapy. Given that up to two-thirds of patients with HPV-driven OPC are either current or ex-smokers at diagnosis, this issue warrants further investigations [6,9–11]. The main purpose of this study is to characterise the mutational profile of HPV-positive OPC by smoking status. We hypothesise a higher frequency of mutations affecting key oncogenes and/or tumour suppressor genes among smokers.

2. Methods

2.1. Patients and tumour samples

Tumour samples from 80 patients with OPC were retrieved from the Gustave Roussy Cancer Center tissue bank. All samples were collected before treatment. Patients were divided into 2 categories according to their tobacco consumption: smokers comprised former and current smokers and non-smokers included individuals' without any smoking history. Clinical notes and pathological reports of each patient were retrospectively analysed by the investigators. Our Institutional Review Board approved the study protocol, and all patients provided written informed consent.

2.2. Preparation of sections from FFPE tumour tissue blocks

Paraffin sections were prepared according to the sandwich method. That is to say the first and last sections were stained by haematoxylin and eosin to check the tumour cell content. The remaining 4 μm sections were used to perform HPV testing and DNA extraction for targeted next-generation sequencing.

2.3. DNA extraction

The DNA of 80 samples were extracted from four 10 μm FFPE pellets using DNeasy tissue kit (Qiagen, Valencia, CA, USA) according to manufacturer's instructions.

2.4. HPV testing

HPV status was determined by p16 immunohistochemistry (CINtec p16 Histology Kit, Roche mtm laboratories AG, Heidelberg, Germany) and detection of HPV DNA according to the manufacturer instructions (INNO-LiPA HPV Genotyping Extra II kit, Fujirebio). Samples were considered to be HPV-driven if both assays were positive. The detailed protocols are described in the Supplementary data (Supplementary Data File No 1).

2.5. Targeted next-generation sequencing

The effect of tobacco on the upper aero digestive tract mucosal lining has been extensively studied, and the most frequently mutated genes in head and neck squamous cell carcinomas (HNSCC) induced by tobacco are well known [12-14]. In the present study, we have assumed that some of these mutations may also be found in HPV-positive smoking patients and could potentially negatively impact their prognosis. Consequently, we have designed a targeted next-generation sequencing (tNGS) panel covering 39 oncogenes or tumour suppressor genes that are recurrently mutated in HNSCC caused by tobacco consumption (Supplementary Data File No 2). These genes were selected based on bibliographic research [12–13]. Library preparation, sequencing techniques and bioinformatics pipeline for tNGS analysis have been previously described [15,16]. The tNGS was performed from 20 ng of tumour DNA amplified in two multiplex PCR-based libraries combining 1763 primer pairs according to Ion AmpliSeq™ workflow and followed by Ion Torrent sequencing (ThermoFisher Scientific, Courtaboeuf, France). Variant calling was performed with Torrent Suite™ software, variantCaller (v5.x;