



Case Report

Human Papillomavirus in Non-Small-Cell Lung Cancer: The Impact of EGFR Mutations and the Response to Erlotinib[☆]

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ARTICLE INFO

Article history:

Received 1 January 2012

Accepted 2 April 2012

Available online 9 February 2013

Keywords:

Epidemiology

Erlotinib

Human papillomavirus

Non-small-cell lung cancer

Epidermal growth factor receptor mutations

ABSTRACT

It has been suggested that human papillomavirus (HPV) could participate in the development of non-small-cell lung cancer (NSCLC). A higher HPV infection rate has been reported in the NSCLC samples from Asian non-smoker patients, with adenocarcinomas or responders to EGFR tyrosine kinase inhibitors (EGFR-TKI). We explored a potential relationship between EGFR mutation, response to EGFR-TKI and HPV infection in Western NSCLC patients. We retrospectively analyzed 40 NSCLC samples and the impact of age, gender, histology, tobacco habit and sample type. HPV infection rate was 2.5% and it was not statistically modified by any analyzed variable, although the limited sample size did not provide definitive conclusions. The rate of HPV infection in NSCLC should be studied in patients with EGFR mutations or a tendency toward presenting them.

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Virus del papiloma humano en cáncer de pulmón no microcítico. Impacto de mutaciones del EGFR o respuesta a erlotinib

RESUMEN

Se ha sugerido que el virus del papiloma humano (HPV) puede originar cáncer de pulmón no microcítico (NSCLC). La tasa de infección HPV es mayor en NSCLC de asiáticos, no fumadores, con adenocarcinoma o respuesta a inhibidores tirosín-cinasa del receptor del factor de crecimiento epidérmico (EGFR-TKI). Hemos explorado retrospectivamente la relación entre mutaciones EGFR o respuesta a EGFR-TKI e infección HPV en 40 pacientes con NSCLC, considerando el impacto del sexo, edad, subtipo histológico, tabaquismo y tipo de muestra. La tasa de infección HPV fue 2.5%, y no influía ninguna variable analizada, aunque el escaso tamaño muestral no permite conclusiones definitivas. La tasa de infección HPV en NSCLC debería revisarse en pacientes con mutaciones EGFR o tendencia a presentarlas.

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Palabras clave:

Epidemiología

Erlotinib

Virus del papiloma humano

Cáncer de pulmón no microcítico

Mutaciones del receptor del factor de crecimiento epidérmico

Introduction

Non-small-cell lung cancer (NSCLC) is the most frequent and lethal tumor in our setting. Tobacco smoke is the cause of more than 90% of cases, but it also appears in unexposed. This implicates the existence of other etiologic factors. In 1979, Syrjänen¹ suggested

that human papillomavirus (HPV) DNA, associated with the development of other cancers, could participate in the development of NSCLC.

The rate of HPV infection in NSCLC depends on multiple variables and can increase with the response to Gefitinib, an endothelial growth factor receptor-tyrosine kinase inhibitor (EGFR-TKI) mainly active in mutated EGFR. The relationship between HPV infection and the presence of EGFR mutations in NSCLC has never been researched, although both phenomena coincide in similar patients: Asians, women, non-smokers, those with adenocarcinomas (ADC) and Gefitinib responders. In order to explore this possibility and to confirm the viability of the project and that the initial results support those in the literature, we retrospectively analyzed 40

[☆] Please cite this article as: Márquez-Medina D, et al. Virus del papiloma humano en cáncer de pulmón no microcítico. Impacto de mutaciones del EGFR o respuesta a erlotinib. Arch Bronconeumol. 2013;49:79–81.

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Table 1
Percentage of HPV Infection in NSCLC Samples According to the Variables of Our Sample.

Sex $P=$.311	Males 20 (0%)	Females 20 (5%)	Non-smokers 20 (5%)
Mean age (range) $P=$.263	62 (39–80)	62 (38–80)	
Smoking $P=$.599	Active smokers 11 (0%)	Light smokers 2 (0%)	Ex-smokers 7 (0%)
Sample type $P=$.366	Biopsy in paraffin 12 (8.33%)	Cell button in paraffin 14 (0%)	Cytology 14 (0%)
Histology $P=$.1	Squamous 5 (0%)	Adenocarcinoma 23 (0%)	BAC 4 (2.5%)
EGFR mutation $P=$.685	Positive 11 (0%)	Negative 23 (4.34%)	Unknown 6 (0%)
Progression-free survival after Erlotinib $P=$.929	>120 days 17 (5.88%)	<120 days 11 (0%)	Progression 12 (0%)
Response to Erlotinib $P=$.659	Partial 11 (9.1%)	Stabilization 5 (0%)	

BAC: adenocarcinoma with areas of bronchioloalveolar carcinoma; EGFR: epidermal growth factor receptor.

NSCLC samples treated in our center between April/2004 and September/2010.

Clinical Observations

Our series (Table 1) includes 20 males and 20 females, all Caucasian and aged 38–80. Thirteen were smokers, 2 smoked fewer than 5 cigarettes/day, 7 were ex-smokers and 20 were never-smokers. The samples were 12 biopsies in paraffin, 14 cell buttons obtained by fine-needle aspiration (FNA) and preserved in paraffin and 14 cytology samples obtained by FNA. We had 5 squamous carcinomas, 23 ADC, 4 ADC with bronchioloalveolar carcinoma (BAC) and 8 unspecified NSCLC. The EGFR status was unknown for 6 samples, 23 had native EGFR and 11 sensitizing mutations: 9 e19(p.746-750del), 1 e19(L747-A750del PIns), 1 e19(L746-A752del VIns), 1 e19(c.2240-2257del), 1 e18(p.G719R), 1 e18(E709A; G719R), 1 e18(E709G) and e21(L858R) and 1 e21(L858R). No resistance mutations were detected.² Twenty-eight cases received Erlotinib, another EGFR-TKI that is more effective on mutated EGFR.

DNA of the samples was extracted with DNeasy tissue kit (Qiagen GmbH, Hilden, Germany) and 37 HPV genotypes were amplified (including 13 varieties with high oncogenicity) with the Linear Array HPV genotyping test (Roche, Switzerland). We assessed the impact of several variables on the rate of HPV infection with either the Fisher or Chi-squared tests.

The incidence of HPV infection in the series was limited. It was detected in 1 out of 40 samples (62-year-old woman, non-smoker, with lung ADC and areas of BAC, native EGFR, responsive to Erlotinib for 7 months). No variables influenced the percentage of infection, although this is conditioned by the size of the series (Table 1). Neither sex (0% males vs 5% females, $P=$.31) nor age (younger or older than 65, $P=$.26) showed an influence. There were no differences among smokers, mild-smokers, ex-smokers or non-smokers ($P=$.56), between biopsy, cell button or cytology ($P=$.36) or between squamous-cell carcinomas, ADC with or without BAC or NSCLC without defined histology ($P=$.1). There were no differences between samples with EGFR mutation, native EGFR or unstudied ($P=$.68), or related with response ($P=$.66) or progression-free survival with Erlotinib ($P=$.93). The limited sample could not confirm differences between response to Erlotinib of NSCLC with the mutation or without it.

Discussion

The main limitation of our study is the small sample size ($No.=$ 40) that does not allow us to draw conclusions about the incidence and association of HPV infection in NSCLC in a Mediterranean population. But our objective was merely to explore the association between HPV infection and presence of EGFR mutations, validate the technique and verify whether the preliminary data supported reports in the literature in order to extend the series in future research. Meanwhile, this and other manuscripts with a similar or smaller number of cases^{3–5} spark discussion and are the first to directly analyze the relationship between Erlotinib sensitivity, presence of EGFR mutations and HPV infection in NSCLC.

HPV infection in NSCLC depends on geographic area. A meta-analysis of 37 studies⁶ ($No.=$ 2435) situates its incidence between 1% and 78.3%, with a mean of 7.1% in western countries and 20.4% in Asia. Another meta-analysis⁷ ($No.=$ 4508) reduces the global rate of infection from 24.5% to 16% in Europe and North America and increases it to 35.7% in Asia. The low rate of HPV infection (0.5%) described in 399 Italian patients with NSCLC⁸ coincides with ours (2.5%), although this would need to be confirmed in more patients.

HPV infection also depends on the sample analyzed. A Canadian study⁹ (No.=1241) and a Croatian study¹⁰ (No.=84) also found no differences between NSCLC and healthy control subjects when comparing bronchial suction or serum samples, but there was evidence of difference in the biopsies. Two Italian studies^{4,11} reported infection rates of 21% and 14%, respectively, when they analyzed 21 paraffin blocks and 95 fresh samples of NSCLC. And the Health Institute in Rome¹² reported 21.7% HPV infection in 2468 NSCLC biopsies.

HPV is found in tumor cells and not in healthy neighboring cells,¹³ and it is therefore more difficult to find evidence of its DNA in bronchial suction or serum samples with less tumor cellularity. Although all our samples presented tumors, there were no differences between biopsies, cell buttons and cytology samples.

Differences have been reported in HPV infection according to NSCLC histology. A Polish article¹⁴ found no differences between 22 squamous-cell carcinomas, 13 ADC and 5 large-cell carcinomas; meanwhile, a French paper¹⁵ reports a greater incidence of ADC in 31 frozen samples (11% vs 25%). In Asia, a Japanese study⁵ with 36 NSCLC observed no differences between squamous carcinomas and ADC (10% vs 9%); a Chinese study¹⁶ with 72 squamous-cell carcinomas, 37 ADC and 71 healthy control subjects found more HPV in squamous-cell carcinoma (51%, 16%, and 22%, respectively); another Japanese article³ found more HPV in ADC than in squamous carcinomas (30% vs 7%; $P=0.044$); and in other studies,^{17,18} it is 2.3 times higher in women, non-smokers and ADC. In Asian studies,^{13,19} HPV infection in NSCLC is higher in women (57.6% vs 41.1%, $P=0.048$) and non-smokers (10.12 times higher).

For the first time, our study probes the possibility that HPV infection is more frequent in patients with EGFR mutation or those who are sensitive to Erlotinib. It has been published that HPV infection increases in NSCLC patients responders to Gefitinib³ (75% vs 0%) and this mainly acts on mutated EGFR. The most frequent EGFR mutations are in women, Asians, non-smokers and those with ADC, which is the population with greater incidence of HPV.

Conclusion

Our data do not confirm the relationship between HPV infection in NSCLC and presence of EGFR mutations or response to Erlotinib. Nevertheless, the determination technique is viable and this hypothesis merits further research with a more extensive series.

Conflict of Interests

The authors declare having no conflicts of interest.

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