Available online at www.sciencedirect.com

SciVerse ScienceDirect

British Journal of Oral and Maxillofacial Surgery 54 (2016) 198–202

BRITISH Journal of Oral and Maxillofacial Surgery

www.bjoms.com

Association between p53 status, human papillomavirus infection, and overall survival in advanced oral cancer after resection and combination systemic treatment

Tommaso Cutilli [a,*], Pietro Leocata [b], Vincenza Dolo [c], Emma Altobelli [d]

[a] *Head of Maxillofacial Surgery Operative Unit, President of Upgrading School of Maxillofacial Surgery, University of L'Aquila – Italy Department of Life, Health & Environmental Sciences*

[b] *Pathology President of Upgrading School of Anatomic Pathology, University of L'Aquila – Italy Department of Life, Health & Environmental Sciences*

[c] *President of Clinical Pathology Postgraduate School, University of L'Aquila – Italy Department of Life, Health & Environmental Sciences*

[d] *Head of Epidemiology and Biostatistics Unit, University of L'Aquila – Italy Department of Life, Health & Environmental Sciences*

Accepted 12 November 2015
Available online 6 December 2015

Abstract

Our previous study on 75 cases of advanced oral squamous cell carcinomas (SCC) treated by neoadjuvant chemotherapy, radical surgery, and radiotherapy showed that overexpression of p53 of more than 50% indicated a strong probability of genetic mutation, and tumours that are characterised by this p53 pattern respond poorly to treatment and have a poor prognosis (p= 0.0001). We have studied the same cohort of patients retrospectively to investigate the incidence of human papillomavirus-16 (HPV16) infection, the relation to the overexpression or mutation of the p53 gene, and the association with overall survival. There were 57 men and 18 women, mean age 67 (range 57-72) years. HPV16 infectivity was found in 66 patients (88%) - 49/57 men (86%) and 17/18 women (94%). There was no significant difference between the sexes (p=0.32). The cumulative survival of the entire group after a follow-up of 38 months was 26% (SE 6.4). The log rank test indicated that the combination of HPV-16 infectivity and p53mutation was significantly related to prognosis (p=0.000). On the other hand HPV16 infectivity alone was not significantly related to prognosis (p=0.78) The incidence of HPV-16 infection decreased with increasing immune p53 expression (p=0.005), whereas that of the HPV16+p53mutation combination increased with increasing immune p53 expression (p=0.000). The results show the importance of the investigation of HPV and p53 expression to define prognosis in oral SCC.

Keywords: Oral Cancer; Surgery; p53 Status; HPV Infection

Introduction

Oral squamous cell carcinoma (SCC) is the most prevalent cancer of the head and neck with over 500 000 new cases every year worldwide. The stage of disease at diagnosis is associated with 5-year survival. Unfortunately, about two-thirds of patients are diagnosed with stages III-IV advanced cancers that are generally related to a poor prognosis, despite the remarkable progress of surgical techniques (for example, transoral robotic surgery), the widespread introduction of microvascular reconstructions, and the progress in chemotherapy and radiotherapy.[1,2]

TNM classification of oral SCC often does not explain the clinical behaviour of the tumour, and the result is often not predictable. Oncogenetic study is increasingly crucial in the staging of a cancer, because it supplies important data for

* Corresponding author at: Via della Comunità Europea, 13 67100 L'Aquila - Italy; Fax: +39 0862 368547.
E-mail addresses: tommaso.cutilli@cc.univaq.it (T. Cutilli), pleocata@cc.univaq.it (P. Leocata), vincenza.dolo@univaq.it (V. Dolo), emma.altobelli@cc.univaq.it (E. Altobelli).

http://dx.doi.org/10.1016/j.bjoms.2015.11.011

diagnosis and prognosis in addition to the classic factors of screening. These data must be used together to provide the surgeon and the oncological team with important elements for better multidisciplinary therapeutic planning.[3–6]

For many years we have been studying the p53 status in oral SSC and during our current research on the evaluation of the prognostic value of p53 protein for advanced oral cancer, we have verified that p53 overexpression of over 50% indicates a poor prognosis. This pattern is associated with a high probability of mutation of the tumour suppressor gene, which indicates a poor response to combination sequential treatments. This information can be acquired beforehand by routine immunohistochemical analysis.[7,8]

The association between SCC of the head and neck and infection with human papilloma viruses (HPV) has aroused considerable interest.[9] The increasing number of studies and particularly knowledge about the damage caused by high-risk HPV16 on p53 led us to check this HPV type on the same patients who we previously treated for advanced oral cancer by neoadjuvant chemotherapy, radical surgery, and adjuvant radiotherapy.[10,11]

We have therefore made a retrospective study to investigate the high-risk HPV-16 (HR-HPV16) infection and its relation to the overexpression or mutation of the p53 gene.

Here we report the results of this study and illustrate the association between these genes and overall survival after oral SCC.

Patients and methods

We made a further retrospective study of the 75 non-consecutive cases of advanced oral SCC treated in our Maxillofacial Surgery Operative Unit that we reported in our previous paper.[8] They were 57 men and 18 women, mean (range) age 67 (57 -72) years selected from a total of 420 patients admitted with advanced oral SCC from January 1992-January 2012, with the criteria homogeneity of histopathological grading (G2) and combination systemic treatment (neoadjuvant chemotherapy, radical resection, and postoperative radiotherapy) defined by the oncological board. The histopathological analysis was made by the same pathologist. The diagnosis grade G2 was based on moderate cellular differentiation. The patients were all smokers.

The protocol of induction chemotherapy comprised cis-diaminodichloroplatinum (CDDP 20 mg/m^2 given intravenously on days 1-5) and 5-fluorouracil (5-FU 1000 mg/m^2 given by continuous infusion through a volumetric pump 2 ml/hour for 5 days). Staging was repeated after three cycles of chemotherapy by computed tomography and magnetic resonance imaging. The treatment was completed by radical excision and neck dissection, followed by adjuvant radiotherapy. The monoclonal p53-AB (DO-7 clone) was used for the immunohistochemical study. Assessments were made by counting the tumour cells with or without expression/field. DNA extraction and amplification by polymerase chain reaction (PCR) (175, 181 e 189 p53 codons) were used to carry out the genetic biomolecular analysis to detect mutations of p53.

Tumours were staged according to the 2002 American Joint Committee on Cancer sixth edition staging criteria.[12]

We evaluated clinical data including age, sex, site of tumour, TNM classification, histopathological grading, p53 immunohistochemical expression, p53 mutation, response to the neoadjuvant chemotherapy, the adjuvant radiotherapy, and overall postoperative survival.

In the present study we retrospectively searched for HPV infectivity, its correlation to the sex of the patients, the overexpression and mutation of p53, cumulative survival, and prognosis.

HPV infectivity was defined by the presence of koilocytic atypia in the specimens. Paraffin-embedded samples from all 75 patients were evaluated for the presence of HPV DNA using both GP5+/GP6+ consensus PCR and type-specific E6/E7 PCR to detect HPV type 16.

Statistical analysis

Continuous variables are presented as mean (SD). We used survival analysis to estimate overall mortality, and the Kaplan-Meier estimator (the product limit method) to evaluate the probability of 12-month survival in relation to the presence of HPV-16 infectivity and HPV+genp53. Differences between survival curves were assessed using the log rank test.

The product limit method was used to evaluate the probability of 12-month survival in relation to the presence of HPV-16 infectivity and HPV+genp53. Differences between survival curves were assessed using the log rank test.

The association between immune p53 expression and HPV-16 infectivity and between immune p53 expression and the combination of HPV-16+gen53 was evaluated using the chi square test or Wilcoxon's test, as appropriate. Finally, the association between immune p53 expression and genp53 combined with the anatomical site of the tumour was analysed using Wilcoxon's test.

Probabilities of less than 0.05 were accepted as significant. All analyses were carried out using SAS/STAT® 9.2 statistical software (SAS Inc, Cary, NC, USA).

Results

The patients' mean age was 67 (range 57-72) years, and no significant difference was found (p=0.18). HPV-16 infectivity was found in 66 patients (88%) - 49/57 men (86%) and 17/18 women (94%), but there was no significant difference between the sexes (p=0.33). The HPV-16+genp53 combination was found in 30 patients (40%) - 21/57 men (37%) and 9/18 women (50%). Again, there was no significant difference between the sexes (p=0.32).