European Journal of Cancer (2014) 50, 765–773

Available at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejcancer.com

Cancer stem cell enrichment marker CD98: A prognostic factor for survival in patients with human papillomavirus-positive oropharyngeal cancer

Michelle M. Rietbergen [a,1], Sanne R. Martens-de Kemp [a,1], Elisabeth Bloemena [b,c], Birgit I. Witte [d], Arjen Brink [a], Robert J. Baatenburg de Jong [e], C. René Leemans [a], Boudewijn J.M. Braakhuis [a], Ruud H. Brakenhoff [a,*]

[a] Department of Otolaryngology/Head-Neck Surgery, VU University Medical Center, Amsterdam, The Netherlands
[b] Department of Pathology, VU University Medical Center, Amsterdam, The Netherlands
[c] Department of Maxillofacial Surgery/Oral Pathology, VU University Medical Center/Academic Centre for Dentistry, Amsterdam, The Netherlands
[d] Department of Epidemiology and Biostatistics, VU University Medical Center, Amsterdam, The Netherlands
[e] Department of Otolaryngology/Head and Neck Surgery, Erasmus Medical Center, Rotterdam, The Netherlands

Available online 4 December 2013

KEYWORDS
Cancer stem cell
CD98
p16
Human papillomavirus (HPV)
Survival
Oropharyngeal cancer

Abstract ***Purpose:*** Several hypotheses have been proposed to explain the relatively good prognosis of patients with a human papillomavirus (HPV)-positive oropharyngeal squamous cell carcinoma (OPSCC) and one of these is a higher sensitivity to (chemo)radiation. Previous studies have suggested that treatment failure in OPSCC patients is caused by resistance of cancer stem cells (CSCs). The purpose of this study was to evaluate the association between the number of CSCs and prognosis in HPV-positive OPSCC patients.
Experimental design: All OPSCC patients ($n = 711$) treated between 2000 and 2006 in two Dutch university hospitals were included. Presence of HPV in a tumour tissue specimen was tested by p16-immunostaining followed by HPV DNA GP5+/6+polymerase chain reaction (PCR). The presence and intensity of tumour CSC markers CD44 and CD98 were determined by immunohistochemistry and semiquantitative scoring was performed. Overall survival (OS) and progression-free survival (PFS) rates were compared between patients with low and high CD44/CD98 expression in relation to HPV status.
Results: HPV-positive tumours showed a lower percentage of cells with CD44 and CD98 expression than HPV-negative tumours ($p < 0.001$, χ^2-test). Within the group of patients with

* *Corresponding author:* Address: Tumor Biology Section, Department of Otolaryngology/Head-Neck Surgery, VU University Medical Center, PO Box 7057, 1007 MB Amsterdam, The Netherlands. Tel.: +31 20 444 0953; fax: +31 20 44 43688.
E-mail address: rh.brakenhoff@vumc.nl (R.H. Brakenhoff).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.ejca.2013.11.010

HPV-positive OPSCC, a high percentage of CD98-positive tumour cells was associated with a significantly worse 5-year OS and PFS (OS: 36.4% and PFS: 27.3%) compared to patients with a low percentage of CD98-positive cells (OS: 71.9% and PFS: 70.5%, respectively) ($p < 0.001$). ***Conclusions:*** HPV-positive OPSCCs harbour fewer cells expressing the CSC enrichment markers CD44 and CD98. Furthermore, OS and PFS were significantly worse for patients with HPV-positive OPSCC with a high percentage of CD98-positive cells.

1. Introduction

Infection with high-risk human papillomavirus (HPV) is aetiologically linked to the development of head and neck squamous cell carcinomas (HNSCCs), particularly those carcinomas that arise in the oropharyngeal region. HPV-positive oropharyngeal squamous cell carcinomas (OPSCCs) are characterised by an epidemiologic, demographical and clinical profile that deviates from that of HPV-negative OPSCCs [1,2]. The most important difference is related to prognosis, which is markedly better for patients with an HPV-positive tumour compared to those with an HPV-negative tumour. Several hypotheses have been proposed to explain the improved outcome for patients with HPV-positive OPSCC, including an increased sensitivity to radiation and chemotherapy, differences in the role of the host immune system and the absence of field cancerisation [3–6]. In this study, we further evaluated the role of cancer stem cells (CSCs) in HPV-positive OPSCCs.

Previous studies suggest that treatment failure in HNSCC patients might be the consequence of therapy resistance of cancer stem cells (CSCs) [7]. CSCs represent a small subpopulation of tumour cells that maintain tumour growth by fuelling the expansion of the malignant cell population infinitely [8]. CSCs can be distinguished from the bulk of the tumour based on differential expression of protein markers on the cell membrane. A large body of evidence indicates that HNSCC cells expressing high levels of the CD44 antigen possess CSC properties. $CD44^{high}$ HNSCC cells have been shown to initiate tumour growth in mice much more efficiently than $CD44^{low}$ cells, indicating that CSCs are enriched in the $CD44^{high}$ subpopulation of HNSCC [9]. Moreover, a high expression of CD44 seems associated with a poor prognosis in patients with HNSCC [10].

Recently, we examined CD98 as a novel, putative CSC enrichment marker in HNSCC and showed that $CD98^{high}$ cells, in contrast to $CD98^{low}$ cells, are able to generate tumours in immune-deficient mice [11]. Studies in a multitude of cancer types showed a higher CD98 expression in progressive and metastatic tumours, which relates to a poor prognosis [12–17].

Recently, it was shown that a small subpopulation of cells with CSC properties could also be isolated from an HPV-positive head and neck cancer cell line [18]. As patients with an HPV-positive OPSCC respond better to treatment and have a more favourable prognosis compared to patients with an HPV-negative OPSCC, we hypothesised that HPV-positive OPSCCs, might have relatively low levels of CSCs. To test this hypothesis, we performed CD44 and CD98 immunostaining on a cohort of OPSCC patients with known HPV status [19]. Furthermore, we evaluated whether CD44 and CD98 expression could be of potential relevance for predicting treatment outcome in patients with HPV-positive OPSCC.

2. Materials and methods

2.1. Patients and tumour samples

To evaluate CD44/CD98 immunostaining and the relation to HPV, a test cohort was composed, which included 88 fresh-frozen, pre-treatment OPSCC samples of patients treated in the period 2008–2011. Eligible samples included histopathologically confirmed invasive squamous cell carcinoma of the oropharynx (International classification of diseases for Oncology, [ICD-10] codes C019, C051, C052, C090–C099 and C100–C109). These OPSCCs were previously tested for HPV using an HPV E6 mRNA reverse-transcriptase polymerase chain reaction (RT-PCR) [19].

To further study the association between survival and CD44/CD98 expression, all patients ($n = 711$) treated between 2000 and 2006 at two Dutch university hospitals were included. Patients were identified through the Dutch Cancer Registries. Patient characteristics and clinical outcome were obtained from the patient files. HPV detection was performed using pre-treatment formalin-fixed, paraffin-embedded (FFPE) biopsies [20]. A sample was scored HPV-positive based on a positive $p16^{INK4A}$-immunohistochemistry (p16-IHC) and a subsequent positive GP5+/6+ HPV DNA PCR, according to a previously validated algorithm [19]. Approval for this retrospective study was obtained from the Institutional Review Board and the study adheres to the guidelines for proper secondary use of human tissue specimen (www.federa.org).

2.2. Immunohistochemical staining of CD44 and CD98

Formalin-fixed paraffin-embedded sections of HNSCC tumour biopsies were deparaffinised and subjected to Tris/EDTA (10 mM/1 mM, pH 9.0) antigen