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Comparison of clinical, radiological and morphological features including the distribution of HPV E6/E7 oncogenes in resection specimens of oropharyngeal squamous cell carcinoma

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ABSTRACT

Background: Human papillomavirus (HPV)-driven oropharyngeal squamous cell carcinoma (OPSCC) represents a distinct tumour entity in comparison to HPV-negative OPSCC. The clinical, radiological, morphological features and distribution of HPV E6/E7 mRNA were investigated in resected specimens of OPSCC.

Methods: We retrieved formalin-fixed, paraffin-embedded whole section slides from 24 p16/HPV-DNA positive and 18 p16/HPV-DNA negative primary tumours and 16 corresponding metastases in patients with early-stage OPSCC who underwent planned curative or diagnostic primary transoral robotic surgery. A detailed clinicoradiological and histopathological investigation of the tumours was performed along with detection of HPV E6/E7 mRNA by in situ hybridisation.

Results: HPV-driven OPSCC was characterised by non-keratinising morphology and was dominated by a cohesive invasion pattern at the leading edge of the tumour. Dysplastic zones of the squamous epithelium were strictly located in the tonsillar crypts in contrast to HPV-negative OPSCC which predominantly arised from the dysplastic surface epithelium. Thirteen HPV-driven OPSCC invaded through the tonsillar lymphoid compartment and into soft tissue, causing a stromal desmoplastic reaction. HPV mRNA was consistently but inhomogenously expressed in the entire tumour area and in the dysplastic squamous epithelium. There was no HPV expression in the adjacent normal epithelium and in the non-neoplastic tissues.

Conclusions: This study enhances the current understanding of HPV-driven OPSCC. Only tumours that invade through the lymphoid compartment induce a stromal desmoplastic reaction. A consistent but inhomogenous expression of E6 and E7 mRNA was found in tumour and dysplastic areas, emphasizing that the E6/E7 oncogenes are the driving factors in HPV-driven OPSCC.

Introduction

Human papillomavirus (HPV)-driven oropharyngeal squamous cell carcinoma (OPSCC) is acknowledged as a distinct entity in the 4th edition of the WHO/IARC Classification of Head and Neck Tumours and in the 8th edition of the UICC TNM Staging Manual [1,2]. The rationale behind these major changes is based on the numerous studies published during the past two decades, establishing a unique and distinct tumour type of HPV-driven OPSCC in terms of anatomy, pathology and clinical profile. The E6 and E7 oncogenes are considered as the driving factors of HPV-driven OPSCC, and they are essential in distinguishing between non-active HPV infection and transcriptionally active HPV infection [3–6]. HPV E6/E7 mRNA can be visually detected in tumour cells by in situ hybridisation (ISH) using formalin-fixed, paraffin-embedded (FFPE) tissue with high sensitivity and specificity [6–12].

The burden of HPV in the development of OPSCC is apparent in the

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western society, including Denmark, accounting for 62% of patients with OPSCC included from 2011 to 2014 with an incidence rate of 4.5 per 100,000 in 2014 [13–17]. Compared to HPV-negative OPSCC, patients with HPV-driven OPSCC tend to be younger males with markedly better prognosis and clinical outcome despite their advanced level of disease [18–20]. Several on-going clinical trials aim to reduce the treatment-related toxicity and morbidity through minimally invasive surgical techniques or de-escalating radiochemotherapy for patients with p16-positive and/or HPV-positive OPSCC [21].

Current histopathological knowledge of OPSCC is primarily based on information obtained from superficial biopsy material. HPV-driven tumours arise from the tonsillar crypts, and even intraepithelial lesions have metastatic potential due to the fenestrated basement membrane of the crypt epithelium or the presence of intraepithelial vessels [22]. In addition, premalignant and field cancerisation zones in the overlying epithelium are absent (as opposed to cervical cancer) [23–25]. HPV-negative OPSCC is known to arise from the overlying epithelium in the tonsils and from other subsites of the oropharynx including soft palate, uvula and the lateral and posterior walls of the pharynx. HPV-driven OPSCC exhibit a non-keratinising and often basaloid morphology with an invasion pattern consisting of cohesive sheets with no desmoplastic reaction [15,26–29]. However, detailed information about the morphology including the invasion pattern, the localisation of dysplastic zones and potential tumour heterogeneity in relation to p16 immunohistochemistry (IHC), and in particular HPV mRNA expression, is lacking. The increasing use of surgical modalities such as transoral robotic surgery (TORS) allows a unique opportunity to obtain resection specimens of OPSCC. A recent study showed that 12 of 30 patients with early stage OPSCC were upstaged primarily in N-site, but also in T-site classification (UICC TNM Staging Manual, 7th edition [30]) following primary surgery, demonstrating that only histopathological examination allows accurate staging [31].

The current study provides a detailed comparative investigation of resected HPV-driven and HPV-negative OPSCC. We hypothesised that HPV-driven tumours do not display dysplastic zones in the overlying squamous epithelium, are characterised by a cohesive invasion pattern with no associated desmoplastic reaction, and exhibit a homogenous expression of HPV E6 and E7 mRNA.

Material and methods

Patient cohort and selection of tumour samples

We retrieved archived resection specimens from 42 consecutively treated patients with early stage (T1-2, N0-1, UICC TNM Staging Manual, 7th edition) squamous cell carcinoma (SCC) located in the palatine or lingual tonsils. All patients were either primary surgically treated or had a diagnostic resection by primary TORS from September 2014 to May 2017. Thirteen patients were referred to postoperative radiation therapy, and five patients were referred to radiochemotherapy, mainly due to N-stage and extracapsular extension (ECE).

Primary tumour specimens and corresponding metastases were collected for histological review and HPV mRNA ISH. All specimens were previously routinely assessed with p16 IHC and for HPV DNA by PCR according to previous studies [15]. Of the 42 primary tumours, 24 (57%) were both p16 and HPV-DNA positive. Strong and uniform p16-staining (both cytoplasmic and nuclear) in > 75% of tumour cells was classified as p16-positive. The p16 IHC and HPV DNA status determined by PCR was compared with that of HPV mRNA by ISH.

The study was conducted according to the Declaration of Helsinki and was approved by the Regional Scientific Ethics Committee (H-1-2014-033) and the Danish Data Protection Agency.

Assessment of tumour on magnetic resonance imaging (MRI)

Thirty-nine MRI scans were retrieved for reassessment. Three scans were inaccessible from other hospitals. One radiologist (G.S.R) reassessed the scans and compared these to the initial assessment. The reassessment was blinded for HPV status, clinical and histological findings. The majority of MRI scans were performed at our institution using Siemens 1.5 Tesla with a scan protocol including T1-axial, diffusion weighted imaging, T2-axial, T2 TIRM coronal and T1 FAT SAT in three planes after contrast injection. All scans were assessed using T2-axial images for tumour assessment including contrast uptake and involvement of either tongue musculature or the superior pharyngeal constrictor muscle.

Assessment of tumour morphology

All tumours were assessed using haematoxylin and eosin (H&E)-stained slides. The tumours were classified as keratinising, non-keratinising or non-keratinising with maturation as previously described [28,30]. Other SCC variants were classified according to the 4th edition of the WHO/IARC Classification of Tumours. The tumour invasion pattern of the leading edge was characterised as non-cohesive, cohesive or mixed. In addition, we assessed the following histological parameters: largest tumour diameter, depth of invasion, tumour invasion through the tonsillar lymphoid compartment, presence of perineural invasion, vascular invasion and desmoplastic stromal reaction. The surgical margin was based on supplementary resections performed intraoperatively and was classified as previously reported (free margin defined as ≥ 2 mm) [31]. We further assessed the presence of tumours within 2 mm of the resected edges in the main specimens. The measurements of tumour diameter and depth of invasion was performed on H&E stained slides. Corresponding tumour sections were marked on macroscopic tumour photos. Moreover, we assessed the presence of ECE in the lymph node metastasis, defined as macro- or microscopic extension through the capsule.

HPV E6/E7 mRNA detection by in situ hybridisation

HPV E6/E7 mRNA was detected using 6 µm FFPE whole slide sections by using a manual RNAscope 2.5 HD Kit performed at Rigshospitalet, Copenhagen University Hospital, Denmark, and an automated RNAscope VS Reagent Kit performed at Aarhus University Hospital, Denmark (both from Advanced Cell Diagnostics Inc., Hayward, CA). The assays were performed in accordance with the manufacturer's instructions. We performed both assays to compare the HPV assay workflow in the laboratory along with the staining results and quality. The manual assay was performed using the ACD HybEZ™ Hybridisation System (Advanced Cell Diagnostics Inc.), and the automated assay was performed using a Ventana Discovery ULTRA autostaining system (Ventana Medical Systems Inc., Tucson, Arizona). All slides were pre-treated with heat and protease prior to target hybridisation. We used a high-risk HPV-7 cocktail probe for the hybridisation of HPV genotypes: 16, 18, 31, 33, 35, 52, and 58 based on previous genotyping results [15]. We used a negative control probe for the bacterial gene DapB (to assess for background signals) and a positive control probe for common housekeeping gene PPIB (to assess tissue RNA quality). During both assays, we applied RNAscope control slides containing FFPE cultured cell pellets of human HeLA cells. All control slides were reviewed prior to hybridisation with the HPV probe.

Interpretation and grading of staining intensity with HPV E6/E7 mRNA

All slides obtained from primary tumours and metastases were reviewed by a head and neck pathologist and the first author (K.K., H.I.C.), and were scored semi-quantitatively according to the scoring guideline by the manufacturer (RNAscope). Positive HPV staining was