HUMAN PAPILLOMA VIRUS TESTING

What is it and how do we do it?

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H&N CANCERS

- Heterogenous malignancies
- 6th most common worldwide
- 90% are SCC and within this there are two groups
  1. Oropharyngeal SCC: 90%, oropharynx (tongue base, soft palate and tonsils)
  2. Non-oropharyngeal SCC – oral cavity, hypopharynx and larynx

RISK FACTORS

- Tobacco – smoking, chewing, snuff
- Alcohol
- HPV
- Chemicals/ionising radiation

HPV

57-70% of oropharyngeal SCCs are HPV+

A much lesser proportion of non-oropharyngeal SCCs are HPV+

So when ‘oral SCC’ is referenced by a patient this is non-specific...

ORAL SQUAMOUS CELL CARCINOMA

These carcinomas can be divided broadly into two groups:

1. OROPHARYNX – HPV

2. ORAL CAVITY – tobacco & alcohol

Tumours within the oropharynx may be either HPV positive or negative and morphologically the appearances are distinctive in those that have been caused by HPV, but why make this distinction?

And what does p16 have to do with it?

p16

- HPV-associated oropharyngeal squamous cell carcinoma is characterised by the expression of the E6 and E7 viral oncoproteins
- E6 oncoprotein degrades p53 at the protein level and consequently, normal p53 response to DNA damage (such as G1 cell cycle arrest or induction of apoptosis) is impaired, resulting in a higher susceptibility to genomic instability
- The E7 oncoprotein degrades/inactivates retinoblastoma tumour suppressor gene product (pRb), preventing it from binding to the E2F transcription factor and thereby promoting cell cycle progression
- As pRb normally downregulates p16 expression, the functional inactivation of pRb results in overexpression of p16

p16

- Thus HPV-associated oropharyngeal squamous cell carcinoma shows nuclear and cytoplasmatic p16-overexpression, which is predominantly absent in HPV-negative oropharyngeal squamous cell carcinoma

- According to the molecular model for tobacco-induced HNSCC, p53 mutation/p16 inactivation is the most frequent event and is also present in early lesions (dysplasia)

- Hence, p16 positivity is regarded as a reliable surrogate marker for E7 ocoprotein expression/HPV-related oropharyngeal carcinoma

WHAT DOES THIS MEAN FOR PATIENTS?

- In a cohort of 107 oropharyngeal squamous cell carcinomas three distinct classes were identified by Weinberger et al 2006:

  Class I: HPV16-negative tumours (39%)

  Class II: HPV16-positive tumours that were p16-negative (23%)

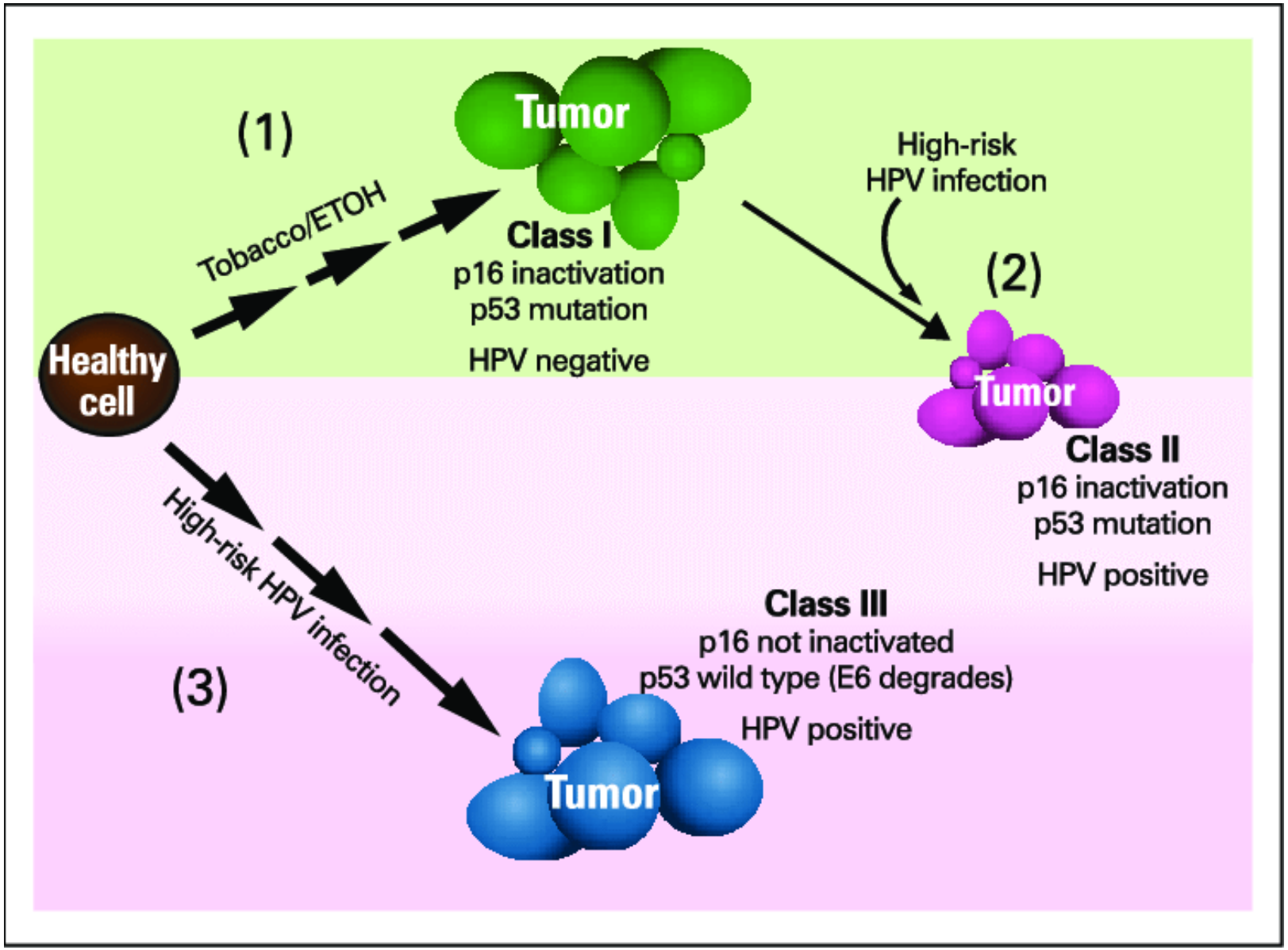
  Class III: HPV16-positive tumours that were p16-positive as well (37%)

- Survival 20%, 18% and 79% respectively

- Only tumours in class III showed significantly lower expression of p53 and pRb, which was not the case in the other classes

- Taken together, these results indicated that simple p16 immunohistochemistry is sufficient for determination of the subset of patients (class III) with biologically relevant, HPV-induced oropharyngeal squamous cell carcinoma

ILLUSTRATION



OTHER STUDIES

- Fischer et al (2010) reported the results of a single institution retrospective study investigating the prognostic significance of p16-expression among 102 assessable patients with oropharyngeal squamous cell carcinoma treated with either radiotherapy or surgery in the early stages (stages I–II) or with combined modality treatment in the more advanced stages (stages III–IV)

- The authors concluded that p16 was the most relevant prognostic factor in oropharyngeal squamous cell carcinoma and that this factor should be considered for inclusion into the official staging system of HNSCC

PATHOLOGY/PROGNOSIS

- HPV-associated carcinomas are usually non-keratinising, arise in the tonsils or tongue base, and evidence consistently shows these tend to have ***better overall and disease free survivals than non-HPV associated carcinomas.***

PATHOLOGY

Thus, within the OROPHARYNGEAL group, pathologists can subdivide into:

1. HPV +
    - usually tonsil & tongue base
    - p16 +
    - non-keratinising
    - not graded

2. HPV –
    - usually soft palate
    - p16 –
    - keratinising
    - graded (well, moderate, poor)

HOW?

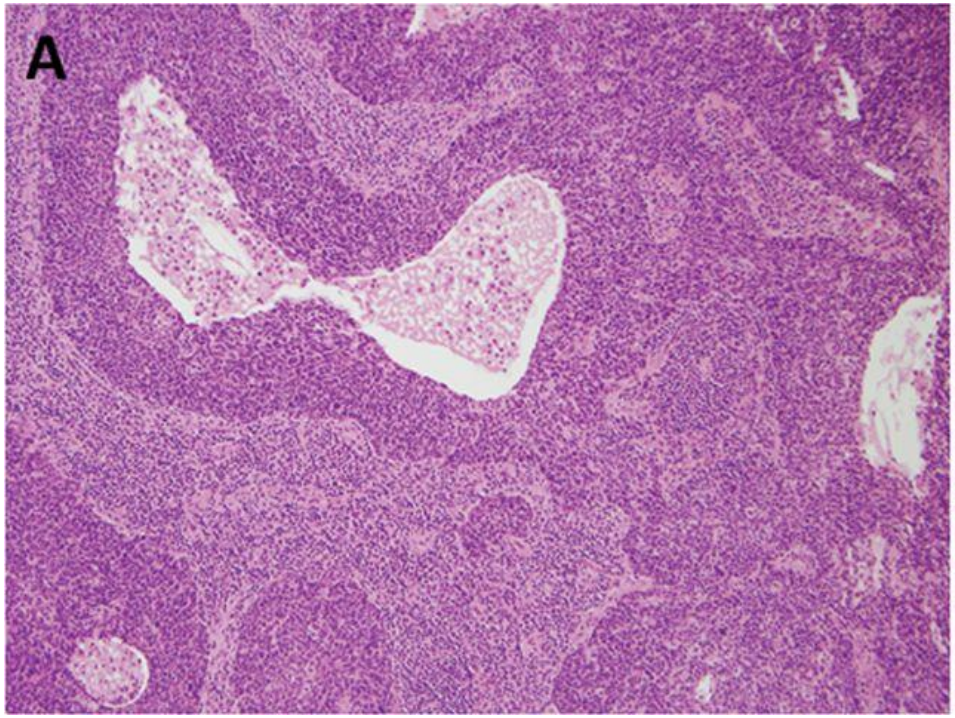
- As the HPV status will inform prognosis, staging and management, it is good practice to make this determination on all oropharyngeal SCCs, whether the morphology is typical or not

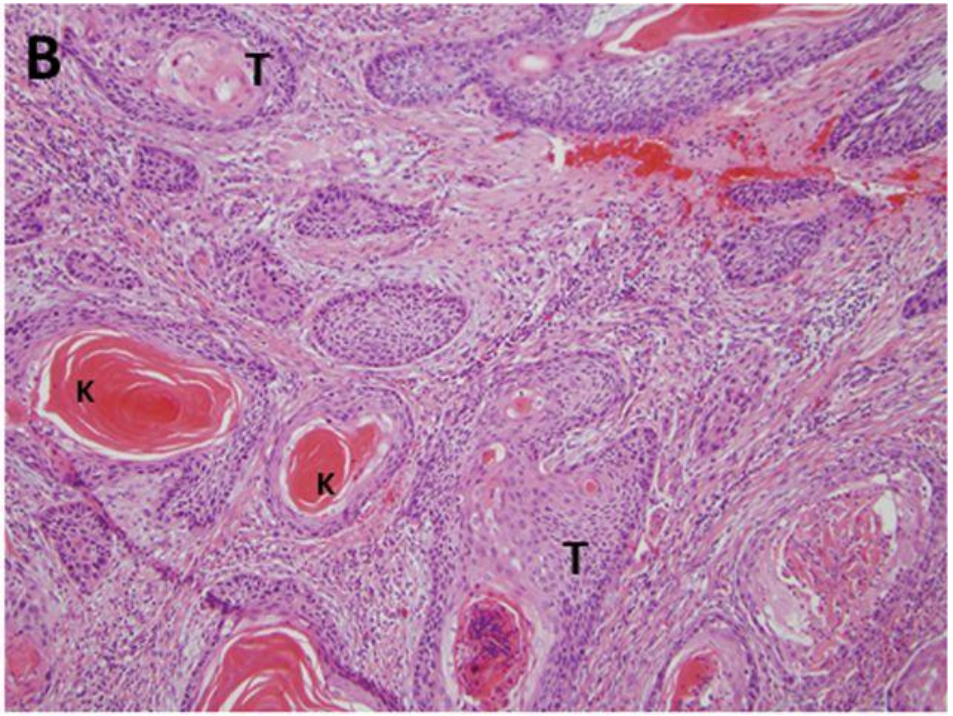
- Overexpression of p16 by ICC is a useful screening method and expression of p16 in >70% malignant cells is considered positive

- p16-negative cases are almost certainly not HPV-associated (or at least not biologically significantly relevant, i.e. the class II tumours referenced previously: p16 inactivation/p53 mutation + HPV)

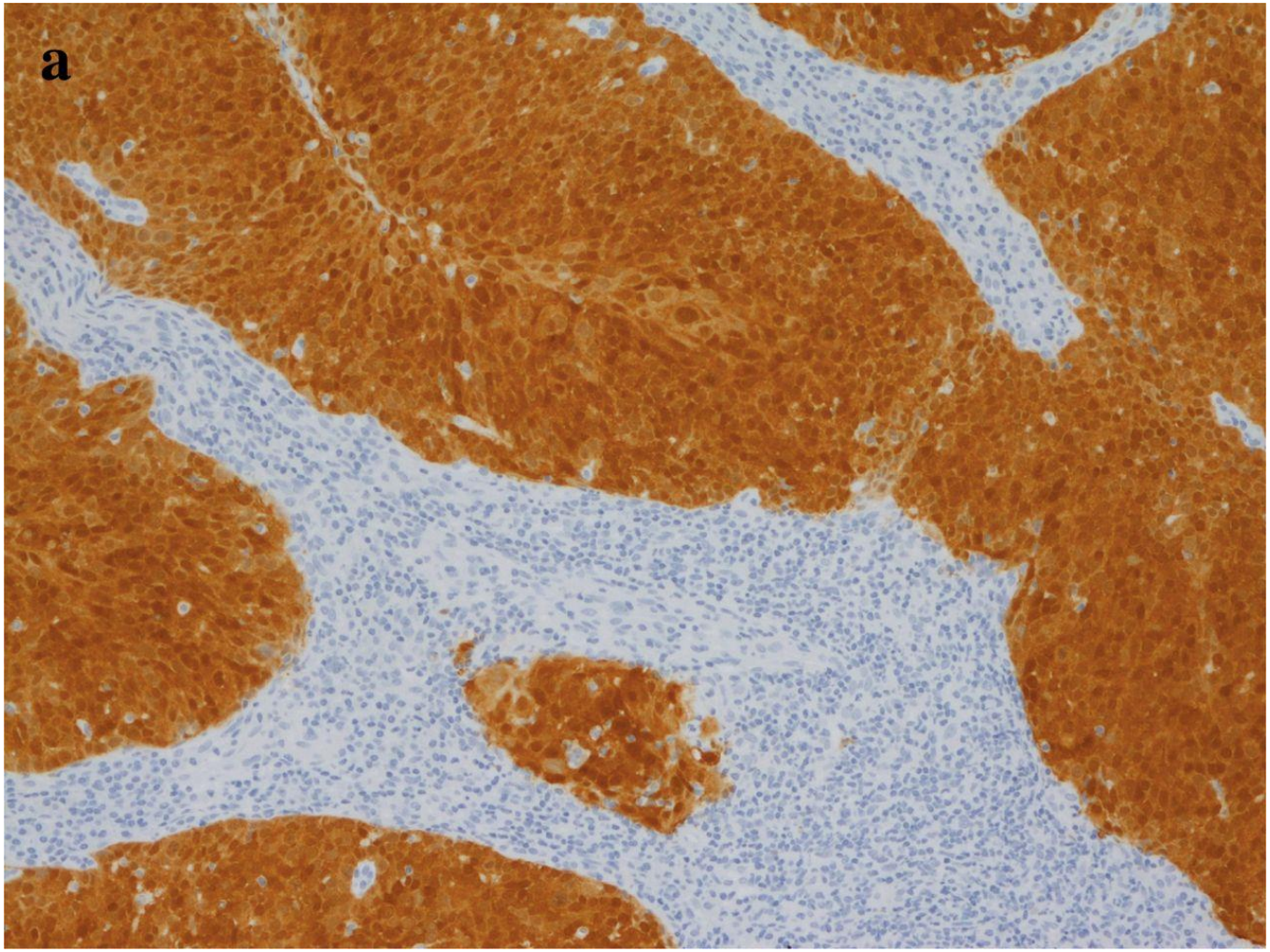
- The ICC is relatively quick, and in the West of Scotland, oropharyngeal carcinomas have the presence of HPV genotype determined by PCR in the Human Papillomavirus Reference Laboratory in Edinburgh

HISTOLOGY





P16+



FINAL DIAGNOSIS

- Following p16 and HPV PCR oropharyngeal SCC can be classified as

  1. OPSCC, HPV associated (not graded)

  2. OPSCC, non-HPV associated (graded)

TAKE HOME MESSAGE

p16 immunohistochemical expression appears to be a reliable surrogate marker for clinically and biologically relevant HPV infection in cases of oropharyngeal squamous cell carcinoma

- Ramqvist T, Dalianis T. An Epidemic of Oropharyngeal Squamous Cell Carcinoma (OSCC) Due to Human Papillomavirus (HPV) Infection and Aspects of Treatment and Prevention. Anticancer Res 2011;31:1515–1519.

- Mehanna H, Jones TM, Gregoire V, Ang KK. Oropharyngeal carcinoma related to human papillomavirus. BMJ 2010;340:c1439.

- Posner MR, Lorch JH, Goloubeva O, Tan M, Schumaker LM, Sarlis NJ et al. Survival and human papillomavirus in oropharynx cancer in TAX 324: a subset analysis from an international phase III trial. Ann Oncol 2011;22:1071–1077.

FIN

Questions?