[1]*Department of Histopathology, Tallaght Hospital, Dublin,Ireland,* [2]*Department of Surgery, Tallaght Hospital, Dublin, Ireland, and* [3]*Department of Histopathology, The Christie NHS Foundation Trust, Manchester, UK*

1. Tang J, He L, Long Z, Wei J. Phyllodes tumor of the verumontanum: a case report. *Diagn. Pathol.* 2015; **10**; 69.
2. Bostwick DG, Hossain D, Qian J *et al.* Phyllodes tumor of the prostate: long-term followup study of 23 cases. *J. Urol.* 2004; **172**; 894–899.
3. Oliveira PA, Colaco AA, Palmeira CA, De la Cruz PL, Lopes CA. A phyllodes tumor of the urinary bladder in a rat. *Exp. Oncol.* 2007; **29**; 159–161.
4. Chen WH, Cheng SP, Tzen CY *et al.* Surgical treatment of phyllodes tumors of the breast: retrospective review of 172 cases. *J. Surg. Oncol.* 2005; **91**; 185–194.
5. Watanabe M, Yamada Y, Kato H *et al.* Malignant phyllodes tumor of the prostate: retrospective review of specimens obtained by sequential transurethral resection. *Pathol. Int.* 2002; **52**; 777–783.
6. Xu LW, Wu HY, Yu YL, Zhang ZG, Li GH. Large phyllodes tumour of the seminal vesicle: case report and literature review. *J. Int. Med. Res.* 2010; **38**; 1861–1867.

H3K27me3 immunostaining for sex determination in the context of presumed tissue misidentification

DOI: 10.1111/his.13364

© 2017 John Wiley & Sons Ltd

Sir: We have read with great interest the correspondence by Dr Crotty and colleagues[1] on the use of X inactive specific transcript (non-protein coding) (XIST) chromogenic *in-situ* hybridisation (ISH) for sex determination in the context of presumed tissue misidentification.

The authors refer to histone H3 trimethyl K27 (H3K27me3) immunostaining, which has been reported to label the inactivated X chromosome[2] and therefore may serve the same purpose as XIST ISH, i.e. an *in-situ* analysis of sex. The mentioned study by Dr Schaefer and colleagues beautifully describes the morphological phenomenon and discusses the underlying biology, but does not assess formally the applicability of H3K27me3 immunostaining for sex determination in a routine setting. We have therefore sought to close this gap by analysing a variety of neoplastic and non-neoplastic tissues (Table 1) in a blinded fashion. H3K27me3 immunohistochemistry was performed on a Leica Bond III immunostainer with a rabbit monoclonal antibody [clone C36B11 at 1:200 dilution with ethylenediamine tetraacetic acid (EDTA)-based antigen retrieval; Cell Signaling, Danvers, MA, USA]. Given that in the context of presumed misidentification the tissue fragments of interest are usually small, we included full histological slides and tissue microarrays (TMA; 600 μm core diameter) of the same neoplastic and non-neoplastic tissue types.

Table 1. Overview of tissues analysed by H3K27me3 immunostaining for sex determination. Autopsy tissue included heart, lung, thyroid, liver, spleen, brain, skeletal muscle and skin

	Male	Female
Normal autopsy tissue – whole slide	19	11
Normal patient tissue – TMA	18	12
Neoplastic – whole slide (glioma)	39	26
Neoplastic – tissue microarray (diffuse glioma)	15	19

TMA, Tissue microarray; H3K27me3, X inactive specific transcript (non-protein coding).

Sex prediction was correct in all 60 non-neoplastic tissues (full slides and TMA cores) in all 34 TMA glioma samples and in 64 of 65 full slides from gliomas. The only tumour for which sex was not predicted correctly derived from a female patient and lacked Barr body staining, both in tumour cells and non-neoplastic cells. Actual tissue misidentification in this case could be ruled out, as microsatellite analysis had been performed in order to test for 1p19q co-deletion.

In summary, H3K27me3 immunohistochemistry predicted sex accurately in 158 of 159 [99.37%; 95% confidence interval (CI) by the modified Wald method: 0.9617 to >0.9999%], which is not significantly different ($P = 1.0000$ by Fisher's exact test) from the results reported by Dr Crotty and colleagues for XIST ISH (268 of 268; 100; 95% CI: 0.9830–1.0000). In the vast majority of samples, sex determination was extremely straightforward and assessed correctly within seconds. In particular, in many female tissues the presence of Barr body-pattern staining was already obvious at medium-power magnification. In more challenging cases, we found the following features useful to distinguish true Barr bodies from non-specific chromatin condensations: Barr bodies were usually single per nucleus, well-defined,

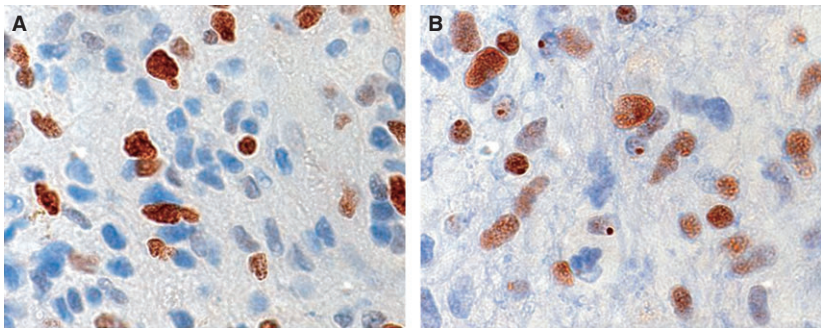

Figure 1. Representative photomicrographs of histone H3 trimethyl K27 (H3K27me3)-stained slides from gliomas of a male (**A**) and a female (**B**) patient.

predominantly near to the nuclear membrane, easiest to identify in cells which lacked any other non-specific staining and present in a higher proportion of cells (Figure 1).

We conclude that H3K27me3 immunostaining can predict sex with high accuracy in both neoplastic and non-neoplastic tissues. Because of its potential in the diagnosis of malignant peripheral nerve sheath tumour[3,4] it will probably be used increasingly in diagnostic routine throughout pathology departments in the future.

Easy availability, cost-effectiveness and rapid turnaround lower the threshold to enter a process of tissue identity analysis. They are therefore critical for its widespread use – and thereby ultimately for patient security – in the context of presumed tissue misidentification. We believe that H3K27me3 immunostaining for sex determination fulfils all these requirements. Furthermore, it offers the advantage of an internal positive control, which may be critical in small tissue fragments of uncertain preservation.

From a broader perspective, we are convinced that a comprehensive approach to tissue misidentification is required: we need to prevent misidentification (i.e. primary prevention in preventive medicine terminology) by appropriate organisational measures at the pre-analytical and analytical level, but also (in the sense of secondary prevention) to have an armamentarium of methods at hand that allows detection of tissue misidentification in a whole variety of scenarios. Such a comprehensive approach will need to include *in-situ* techniques (of which XIST-ISH and H3K27me3 are promising candidates) to serve patient security optimally.

Acknowledgements

The authors thank the staff of the diagnostic immunohistochemistry laboratory and the Translational Research Unit at their department.

Conflict of interest

The authors declare that they have no conflicts of interest.

Joëlle Tschui
Ekkehard Hewer

Institute of Pathology, University of Bern, Bern, Switzerland

1. Crotty R, Arora K, Prasad S, Ting DT, Rivera MN, Deshpande V. X-inactive specific transcript RNA *in-situ* hybridization as a tool for resolving specimen contamination events. *Histopathology* 2017; **71**; 662–665.
2. Schaefer IM, Minkovsky A, Hornick JL. H3K27me3 immunohistochemistry highlights the inactivated X chromosome (Xi) and predicts sex in non-neoplastic tissues. *Histopathology* 2016; **69**; 702–704.
3. Schaefer IM, Fletcher CD, Hornick JL. Loss of H3K27 trimethylation distinguishes malignant peripheral nerve sheath tumors from histologic mimics. *Mod. Pathol.* 2016; **29**; 4–13.
4. Prieto-Granada CN, Wiesner T, Messina JL, Jungbluth AA, Chi P, Antonescu CR. Loss of H3K27me3 expression is a highly sensitive marker for sporadic and radiation-induced MPNST. *Am. J. Surg. Pathol.* 2016; **40**; 479–489.

High molecular weight caldesmon expression in ovarian adult granulosa cell tumour and fibrothecoma

DOI: 10.1111/his.13365

© 2017 John Wiley & Sons Ltd

Sir: High molecular weight caldesmon (H-caldesmon) is a protein that binds to actin and tropomyosin regulating smooth muscle contraction function.[1] It is