Pathology (April 2019) **51(3)**, pp. 253–260

ANATOMICAL PATHOLOGY

Patterns of p53 immunoreactivity in non-neoplastic and neoplastic Barrett's mucosa of the oesophagus: in-depth evaluation in endoscopic mucosal resections

Christopher Toon[1], Benjamin Allanson[2], Connull Leslie[3], Nathan Acott[3], Bob Mirzai[3,6], Spiro Raftopoulos[4], Marian Priyanthi Kumarasinghe[3,5]

[1]*Histopath Diagnostic Specialists, North Ryde, NSW, Australia;* [2]*Department of Tissue Pathology and Diagnostic Oncology, Royal Prince Alfred Hospital, Camperdown, NSW, Australia;* [3]*PathWest Laboratory Medicine, Perth, WA, Australia;* [4]*Department of Gastroenterology, Sir Charles Gairdner Hospital, Perth, WA, Australia;* [5]*School of Pathology and Laboratory Medicine, University of Western Australia, Perth, WA, Australia;* [6]*School of Biomedical Sciences, University of Western Australia, Perth, WA, Australia*

Summary

There is increasing interest in p53 immunohistochemistry as an adjunct to haematoxylin and eosin (H&E) assessment for dysplasia in oesophageal Barrett's mucosa; however, published information on the patterns of staining remains scant. Here, we present descriptions of normal and aberrant p53 staining in non-neoplastic and dysplastic Barrett's mucosa in endoscopic mucosal resections.

A retrospective series of archival endoscopic mucosal resections for biopsy proven dysplasia at our institution were retrieved for this study, comprising 28 sections from 23 patients. p53 immunohistochemistry was performed using an in-house optimised protocol and the staining pattern assessed in H&E confirmed non-neoplastic, dysplastic and neoplastic areas of Barrett's mucosa with regard to individual cell intensity and location of positive cells with respect to gland microanatomy. In non-neoplastic epithelium, normal p53 staining was weak, heterogenous and localised to the crypts. In dysplastic epithelium, p53 overexpression was seen which was of moderate to strong intensity in either a crypt predominant location or diffuse involving crypt and surface epithelium. The crypt predominant pattern was observed more commonly in low grade dysplasia while the diffuse pattern was more commonly seen in high grade dysplasia. In a minority of cases, there was complete loss of p53 staining in dysplastic epithelium and contiguous neoplasia (null phenotype).

p53 immuno-expression in non-neoplastic and dysplastic Barrett's mucosa is distinctive when interpreted with regard to cell intensity and gland microanatomy. We propose that these staining patterns may assist in the interpretation of dysplasia in endoscopic biopsies of Barrett's mucosa.

Key words: Barrett's mucosa; dysplasia; p53 immunohistochemistry.

Received 1 October, revised 21 November, accepted 3 December 2018
Available online 28 February 2019

INTRODUCTION

Surveillance for dysplasia in Barrett's mucosa is critical for the early detection of adenocarcinoma of the tubular oesophagus and gastro-oesophageal junction, a major cause of morbidity and mortality across the globe which appears to be increasing in incidence.[1,2] Importantly, early pre-neoplasia in Barrett's mucosa is amenable to treatment with endoscopic mucosal resection or radiofrequency ablation, minimally invasive procedures with good outcomes.[3,4] Morphological evaluation for dysplasia on haematoxylin and eosin (H&E) stained sections remains the mainstay in the evaluation of endoscopic biopsies of Barrett's mucosa; however, this approach is subject to inter-observer variability, even among experienced gastrointestinal (GIT) pathologists.[5–8]

In recent years, the use of p53 immunohistochemistry as an adjunct to H&E assessment has received increasing interest. Aberrant immunohistochemical staining for p53 (either overexpression or loss) is a biomarker for underlying *TP53* mutation. Over-expression likely reflects stabilisation of an inactivated protein resulting in *in vivo* accumulation, while complete loss of expression likely reflects either a truncating mutation or epigenetic silencing in association with loss of heterozygosity. Mutation or functional inactivation of TP53 is a feature of many tumours,[9] and while the intact protein product is considered to have tumour suppressor function, there is evidence that accumulation of mutant p53 protein may result in both gain-of-function as well as loss-of-function due to inhibition of residual wild-type protein.[10]

Studies in Barrett's neoplasia employing molecular methods have shown expansion of *TP53* mutated clones in multiple biopsies with high grade dysplasia.[11] Whole-exome and whole-genome studies of oesophageal adenocarcinoma have shown *TP53* to be the most frequently mutated gene with more than 70% of samples showing loss of function mutations, where loss appears to be associated with whole-genome doubling and acceleration of tumour progression.[12] There is recent evidence that *TP53* mutations can be detected in early non-dysplastic surveillance biopsies in patients who subsequently progress to high grade dysplasia and adenocarcinoma, with potential for screening paradigms to include this biomarker.[13,14] A number of recent studies have demonstrated the utility of immunohistochemistry in detecting aberrant p53 expression in dysplastic Barrett's mucosa.[15–17]

Print ISSN 0031-3025/Online ISSN 1465-3931
DOI: https://doi.org/10.1016/j.pathol.2018.12.415

Despite accumulating evidence for the utility of p53 immunohistochemistry in distinguishing dysplastic Barrett's mucosa from benign mimics, acceptance for routine testing such as in cases where there is atypia indefinite for dysplasia remains poor among diagnostic pathologists. This is due in large part to two significant hurdles; a lack of guidance for the standardisation of p53 immunohistochemical techniques specifically validated for endoscopic specimens of Barrett's mucosa in the routine diagnostic laboratory; and the fact that p53 immuno-expression can be observed in non-dysplastic as well as dysplastic/neoplastic cells (i.e., p53 immunohistochemistry is not an 'all or none' result).

The aim of this study is to describe the patterns of normal and aberrant p53 expression in endoscopic mucosal resections with Barrett's mucosa harbouring H&E confirmed low and high-grade dysplasia and intramucosal carcinoma, based on an in-house optimised protocol for p53 utilising a commercially available antibody clone. We hope to formulate and recommend a standard approach in the performance and interpretation of p53 immunohistochemistry as an adjunct to routine H&E assessment of Barrett's mucosa.

MATERIALS AND METHODS

A series of 23 endoscopic mucosal resections performed for biopsy proven dysplasia in Barrett's mucosa were retrieved from the hospital pathology department archives at PathWest, QEII Medical Centre and Sir Charles Gairdner Hospital (years 2009–2018 inclusive). One or more tissue blocks from each case with well visualised areas of low and high-grade dysplasia were selected. The H&E sections were re-evaluated by a senior experienced GIT pathologist (MPK) to confirm the presence and extent of low and/or high-grade dysplasia and, where present, intramucosal carcinoma. Consistent with national and international guidelines for routine reporting of Barrett's associated neoplasia, all cases had previously undergone double-reporting with input from at least one pathologist experienced in GIT as part of their diagnostic workup.

Immunohistochemistry for p53 was performed on whole sections using a commercially available mouse monoclonal antibody (clone DO-7; cat no. M7001; Dako, Denmark) on the BenchMark ULTRA platform (Roche, USA). Sections were cut at 4 μm on Matsunami Platinum Pro charged slides (Matsunami Glass Ind. Ltd, Japan), then dried in an oven at 60°C for 60 min. Heat induced epitope retrieval was performed with an alkaline buffer (CC1 pH 8.5; cat no. 950-224; Ventana, USA) for 48 min, followed by incubation with a 1:2000 dilution (Dako diluent; cat no. S08098) of the primary antibody for 32 min at 36°C. The successful reaction was visualised using the Ventana 3 step detection system OptiView (cat no. 950-224). Verification of the successful reaction on each slide was performed with tonsil and appendix as external tissue controls (Assessment Run 38, 2013; NordiQC, Denmark).

In-house optimisation and validation was performed (NA, PK) using appendix and tonsil tissues, as per the recommendation of NordiQC. Parameters of the protocol which were adjusted to achieve the optimal protocol included heat induced epitope retrieval (HIER) time, antibody dilution, antibody incubation time and detection type (with and without amplification). The stained slides were reviewed (PK, NA) and the optimal protocol chosen. As described by NordiQC, the optimal protocol showed a weak to moderate nuclear staining reaction in more than 20% of germinal centre B-cells, and less than 10% of the mantle zone B-cells were demonstrated in the tonsil. In the appendix, dispersed epithelial cells in the basal parts of the crypts showed a weak to moderate nuclear staining reaction, while the luminal epithelial cells were negative. Twenty-three cases were used in the validation study. These included gastrointestinal and neurological cases where the use of p53 was required to aid in diagnosis. The staining results of the validation study were concordant with the expected staining results of the cases used.

For this study, interpretation of p53 staining was based on the assessment of two visual parameters: (1) staining intensity of individual epithelial cells; (2) location of the cells within a stereotypical Barrett's gland (i.e., staining pattern).

Cell staining intensity

Cell staining intensity was assessed as weak (pale yellow or light brown, barely visible), moderate (visible brown) or strong (dark brown including a 'matt finish' quality to the nucleus) and interpreted as follows: weak to moderate = negative; moderate to strong = positive; moderate only = equivocal.

Staining pattern in a Barrett's gland

The location of cells immunoreactive for p53 within a Barrett's gland was noted with respect to following compartments: proliferation zone of crypts, base of crypts, and surface (Fig. 1).

Ethics

This study was performed according to the Declaration of Helsinki and is governed by Sir Charles Gairdner Hospital/Western Australia Health institutional ethics reference no. 2013-153.

RESULTS

Unequivocal areas of Barrett's mucosa with low and/or high-grade dysplasia were present in all selected sections. Squamous epithelium was present in 10 sections. Intramucosal carcinoma was present in 15 sections, ranging from focal to widespread. Areas of low and high grade dysplasia were often present in the same tissue section in varying proportions. A visual representation of the approximate relative proportions of squamous and columnar mucosa (with non-dysplastic and dysplastic/neoplastic Barrett's mucosa) is shown in Fig. 2.

Nuclear localisation of p53 staining was well visualised in all cases, with the weakest intensity (i.e., normal) seen in non-dysplastic glands, and the strongest in dysplastic glands and intramucosal carcinoma. The strongest intensity staining had a 'matt finish' quality reminiscent of dark rosewood or dark brown matt nail polish (Fig. 3). In a few cases, there was complete loss of staining in dysplastic mucosa and contiguous carcinoma (null phenotype).

Subepithelial stromal lymphocytes and basal keratinocytes (where squamous epithelium was present) were well visualised in p53 stained sections and served as useful internal positive controls. Of all normal cells the strongest intensity staining was observed in basal keratinocytes. Non-neoplastic columnar mucosa showed only weak or focally moderate intensity of staining. Lymphocytes showed a spectrum of staining intensity from weak to focally moderate, with the strongest intensity typically weaker than that seen in basal keratinocytes. The smooth muscle cells of the muscularis mucosa and endothelial cells showed only weak focal staining and were essentially non-immunoreactive for p53.

Normal p53 expression (non-dysplastic columnar epithelium) (Fig. 4)

Non-dysplastic and reactive columnar epithelium of Barrett's and gastric type (non-specialised) epithelium showed mainly weak to focally moderate staining in crypts and to a much lesser extent the surface epithelium. Crypt staining was best visualised in the proliferation zones where there was weak to focally moderate intensity staining. Strong intensity staining was not identified in any cell throughout the non-dysplastic gland. Crypt (pyloric gland type) bases were largely devoid of staining. There was cell-to-cell variability in p53 intensity (non-clonal pattern), imparting a heterogeneous visual impression.

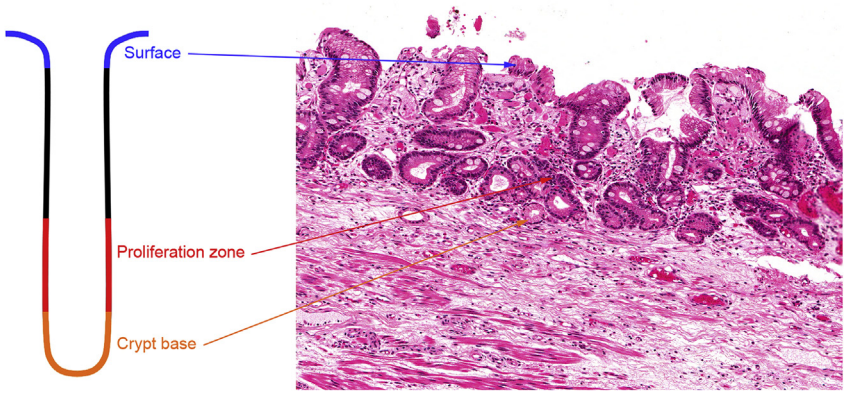

Fig. 1 Diagrammatic representation of a stereotypical Barrett's mucosa gland and corresponding H&E view.

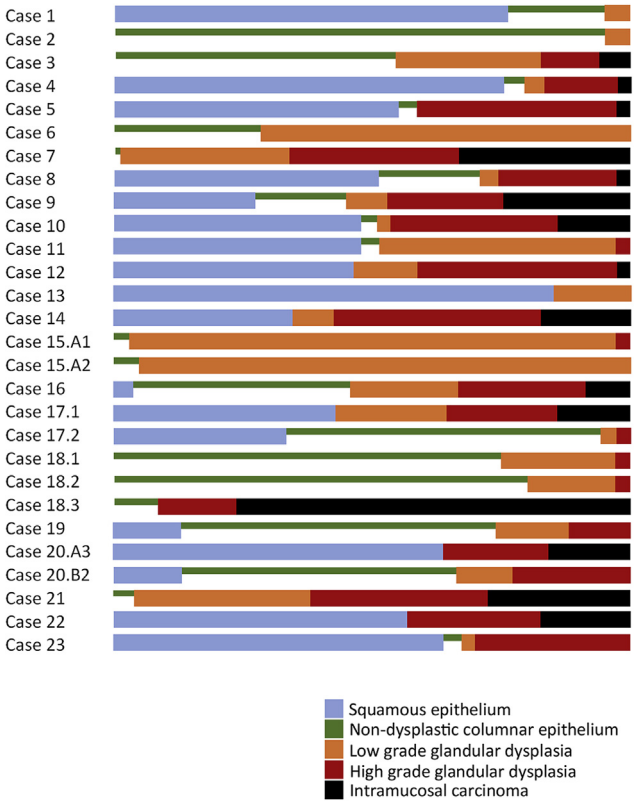

Fig. 2 Relative proportions of squamous epithelium, columnar epithelium (gastric type and Barrett's), dysplastic Barrett's and intramucosal carcinoma in 28 tissue sections from 23 patients (cases), estimated by visual inspection. There was a preponderance of sections with intramucosal carcinoma as endoscopic mucosal resections were more commonly performed following diagnostic confirmation of high grade dysplasia in prior endoscopic biopsies. Low and high grade dysplasia coexisted in many sections.

In reactive metaplastic columnar epithelium, more crypt cells showed moderate intensity staining, corresponding to an expanded proliferation zone. Surface epithelium in metaplastic epithelium was negative for p53 staining.

p53 over-expression in dysplastic Barrett's mucosa (Fig. 5 and 6)

Moderate to strong intensity staining was observed in dysplastic Barrett's mucosa and contiguous intramucosal carcinoma (where present). The cell-to-cell staining intensity was visually homogeneous (clonal pattern). The intense staining was apparent on scanning magnification and was of several magnitudes stronger than background lymphocytes or basal keratinocytes.

With regard to gland microanatomy, staining was either diffuse, involving crypts and surface epithelium, or crypt predominant with minimal to variable involvement of the surface epithelium. Diffuse staining (involving crypt and surface epithelium) was noted in all areas of high grade dysplasia and in a minority (n=2) of low grade dysplasia areas. The majority of low grade dysplasia areas showed a crypt predominant staining pattern.

In summary, two patterns of over-expression were observed: one largely confined to crypt bases, and the other more obvious diffuse staining involving crypt and surface epithelium.

p53 loss of expression (null phenotype) in dysplastic Barrett's mucosa (Fig. 7)

In four sections, there was complete loss of p53 staining (clonal pattern) in low and high grade dysplasia, and contiguous intramucosal carcinoma (where present). This loss of staining was in sharp contrast to normal weak heterogeneous staining of non-dysplastic glands and background positive internal control of subepithelial stromal lymphocytes and basal keratinocytes.

DISCUSSION

Recent studies on p53 immunohistochemistry in Barrett's mucosa have examined the predictive value of p53 for dysplasia as well as in improving inter-observer concordance for histological diagnosis of low and high-grade dysplasia;[18,19] however, few studies have systematically described the patterns of p53 staining in non-neoplastic and neoplastic Barrett's mucosa.

In this study, we show that p53 immunoreactivity in normal and dysplastic/neoplastic Barrett's mucosa is distinctive and can be readily appreciated when assessed using a multi-parametric approach (integrating cell intensity and cell location within a stereotypic Barrett's gland). Our p53 antibody has been extensively published and is a commercially available clone that is widely available in many countries including the USA, UK and continental Europe, facilitating ease of adoption in the diagnostic setting.

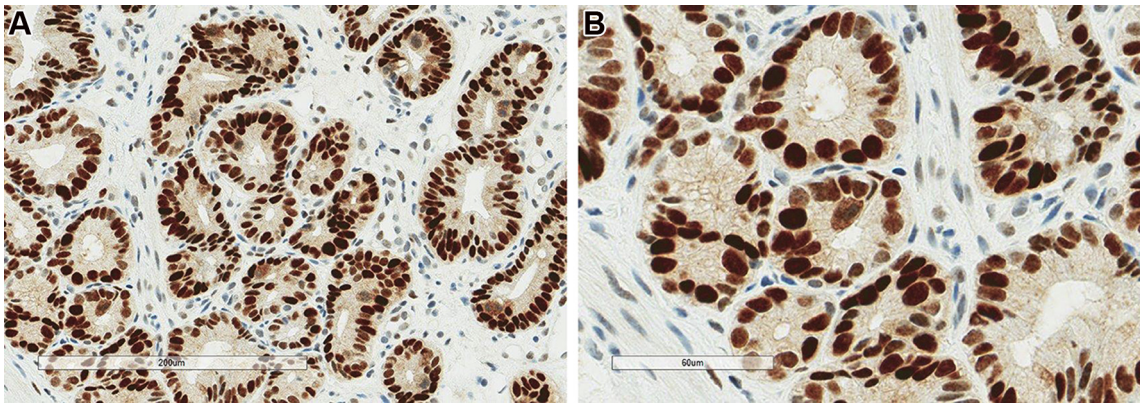

Fig. 3 Typical strong p53 staining with a 'matt finish' quality in dysplastic Barrett's glands, consistent with p53 over-expression. (A) Low power, (B) higher power view. Note the homogeneous staining intensity, indicative of a clonal process.

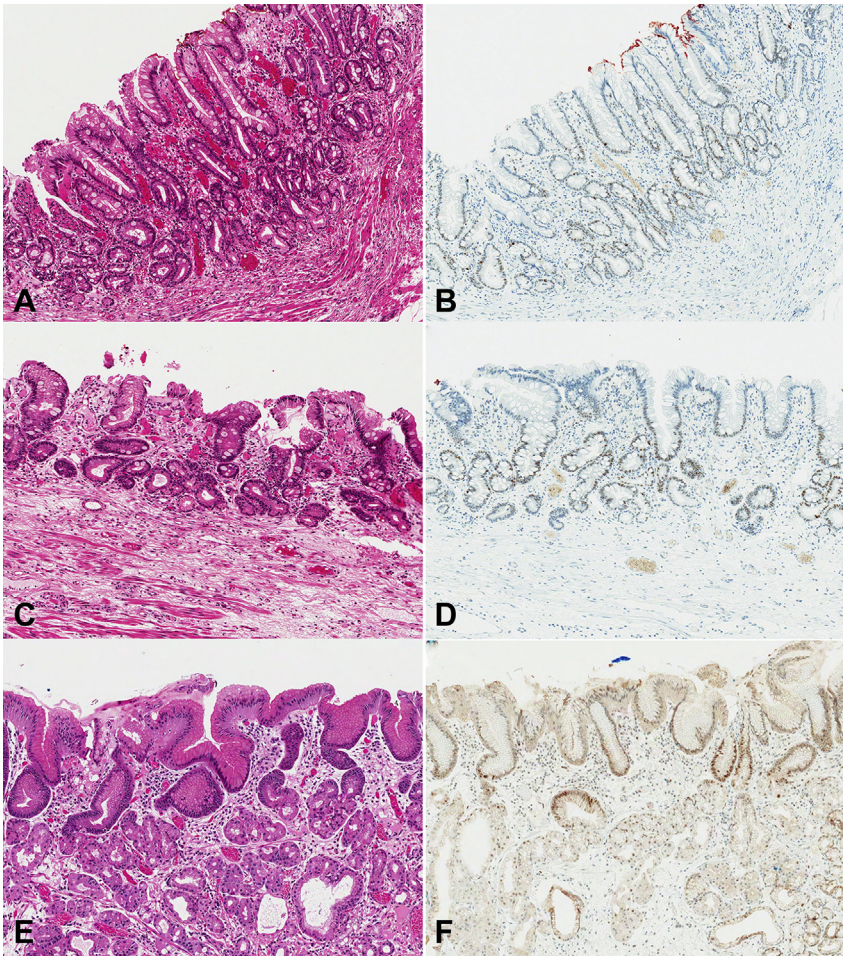

Fig. 4 Normal p53 staining (pattern 1) in non-dysplastic glandular (Barrett's in panels A/B and C/D; gastric type in E/F). Staining intensity was weak to focally moderate, mainly seen in the proliferation zone of crypts. The surface and crypt (pyloric) bases were essentially unstained (non-reactive). There are cell-to-cell variation in staining intensity (non-clonal pattern) imparting a heterogeneous visual impression which could be appreciated on scanning magnification.

The following points merit consideration when interpreting p53 immunoreactivity using an in-house optimised protocol:

1. Normal p53 staining should never show strong intensity. This is typically confined to dysplastic and neoplastic cells. Strong intensity staining has a typical 'matt finish' quality (Fig. 3).
2. Heterogeneous cell-to-cell variability in p53 staining is a non-clonal feature which underpins normal staining, including in expanded proliferation zones of metaplastic columnar epithelium.
3. In dysplastic Barrett's mucosa, the (pyloric) crypt bases are typically obliterated, resulting in homogeneous crypt staining for p53.
4. Background lymphocytes and basal keratinocytes are useful not just as internal controls; they serve as a visual guide to the spectrum of staining intensities one should expect in normal p53 staining. With null phenotype

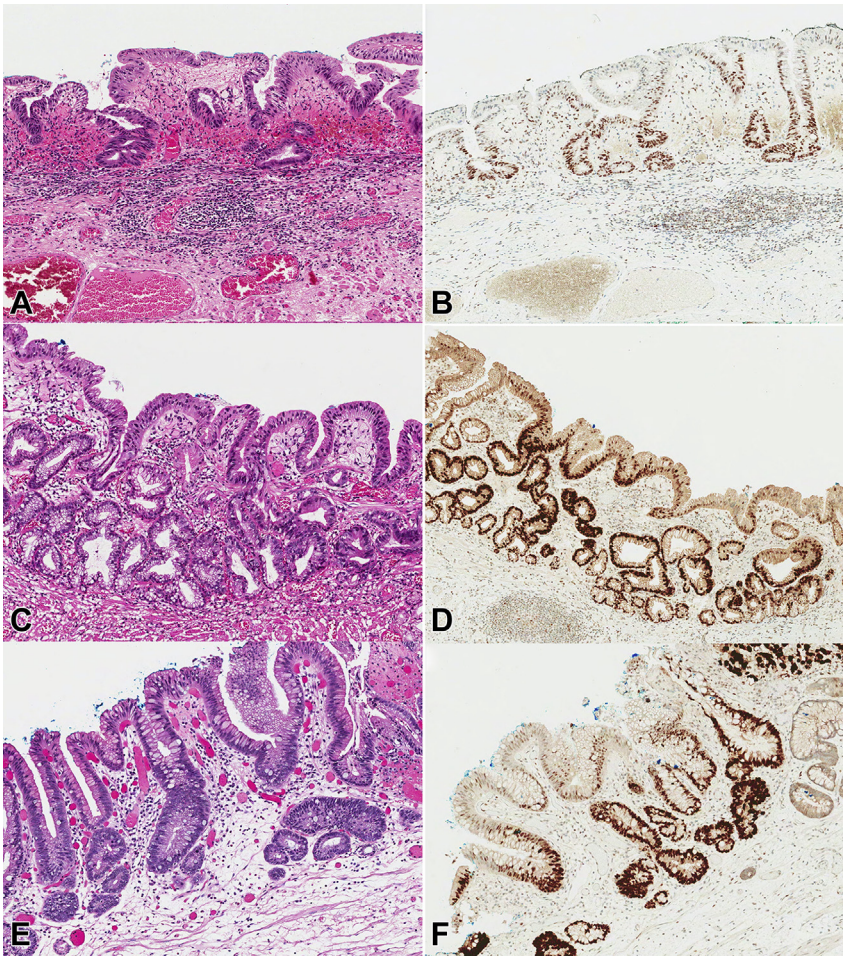

Fig. 5 (A–F) p53 over-expression with a crypt predominant pattern (pattern 2) in three cases within low grade dysplastic Barrett's mucosa. (D) Variable surface staining (approaching that of pattern 3) could be seen.

dysplasia/neoplasia, these background cells provide a striking visual contrast.

5. Squamous islands, which are due to incomplete visualisation of the squamous epithelium due to tangential sectioning where only the basal layers are seen, are not infrequently noted in endoscopic biopsies. These islands can show strong p53 staining (a normal feature previously noted), giving a 'false' impression of aberrant p53 expression if interpreted as a crypt base. Assessment of p53 staining should always be performed in conjunction with H&E levels.

We believe that the crypt predominant p53 staining pattern noted in low grade dysplastic Barrett's mucosa is an artefact of two dimensional views of a gland which exists in three dimensional space, where there is stochastic displacement of non-neoplastic epithelial cells by the clonal proliferation of dysplastic cells from the proliferation zones, rather than an indication of true crypt only dysplasia. Epithelial cycling in a Barrett's gland is a bi-directional process which originates in the proliferation zones of crypts.[20,21] Therefore, it follows that crypt and surface colonisation by dysplasia occurs in tandem but results in uneven colonisation of the surface compared to the crypts due to differences in gland microanatomy. In a typical endoscopic mucosal resection specimen, inspection of the breadth and length of a given low grade dysplastic area with crypt predominant p53 staining invariably identifies one or more individual dysplastic glands which also show surface p53 staining.

We note that Khan *et al.* have described clonal CDKN2A mutations shared by crypts and surface epithelium of dysplastic Barrett's glands. The authors suggest that crypt dysplasia may be real and due to surface phenotypic maturation by non-terminally differentiated dysplastic progenitor cells.[22]

A small number of cases show loss of p53 expression in dysplastic/neoplastic mucosa. This pattern of aberrant expression is less common than over-expression, but is associated with greater risk of neoplastic progression compared to over-expressed p53 pre-neoplastic lesions.[23–25] For this study, we have not attempted to enumerate the number of cases or sections with patterns 2 and 3 as both patterns tended to be present in varying proportions in a given case or section where areas of low and high grade dysplasia were present, consistent with the dynamic nature in which p53 aberrant expression and the development H&E visible dysplasia and neoplasia occurs in Barrett's mucosa.

We caution interpretation of p53 immunoreactivity in metaplastic columnar epithelium. The expansion of the crypt

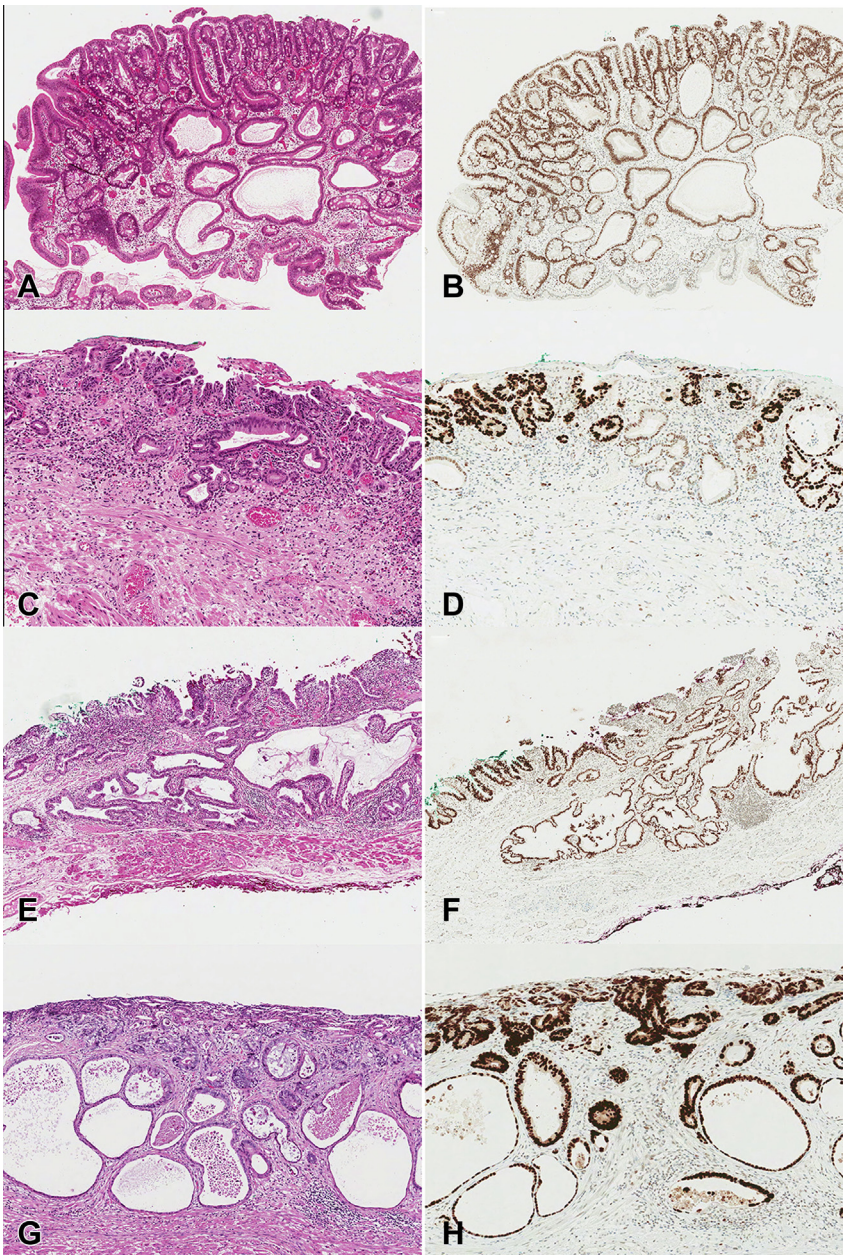

Fig. 6 p53 over-expression in dysplastic Barrett's mucosa with diffuse staining of crypt and surface epithelium (pattern 3). (A,B) A case of low grade dysplasia with this pattern of staining. (C–H) Cases with high grade dysplasia, with contiguous intramucosal carcinoma present in E,F and G,H.

proliferation zone which can be seen in these areas may be associated with moderate intensity staining in more crypt cells compared to the normal p53 staining pattern. However, closer examination typically reveals a heterogenous staining intensity in these cells, and their localisation in the expanded proliferation zone of crypts. In our experience, these areas never showed a 'matt finish' quality to the staining, a feature of p53 over-expression.

We did not assess for interobserver variation of our approach as the aim of this study was to describe and document the patterns of p53 staining in H&E confirmed non-dysplastic and dysplastic areas of Barrett's mucosa. However our multi-parametric approach to assessment of p53 staining is designed to improve objectivity of interpretation. It is our hope that this methodology will form a rational foundation upon which improved reliability in p53 immunohistochemical interpretation can be achieved when assessing for dysplasia in endoscopic biopsies of Barrett's mucosa.

In summary, we describe in this study four patterns of p53 immunoreactivity in the context of H&E confirmed non-neoplastic and dysplastic/neoplastic Barrett's mucosa using endoscopic mucosal resection specimens (Table 1, Fig. 8). Normal and aberrant p53 immunoreactivity appear distinctive when described based on individual cell intensity and location of positive staining cells in the Barrett's gland. In dysplastic Barrett's mucosa with p53 over-expression, crypt predominant staining is more commonly seen in low grade dysplasia, while diffuse crypt and surface staining is characteristic of high grade dysplasia. Contiguous intramucosal carcinoma shows identical p53 staining.

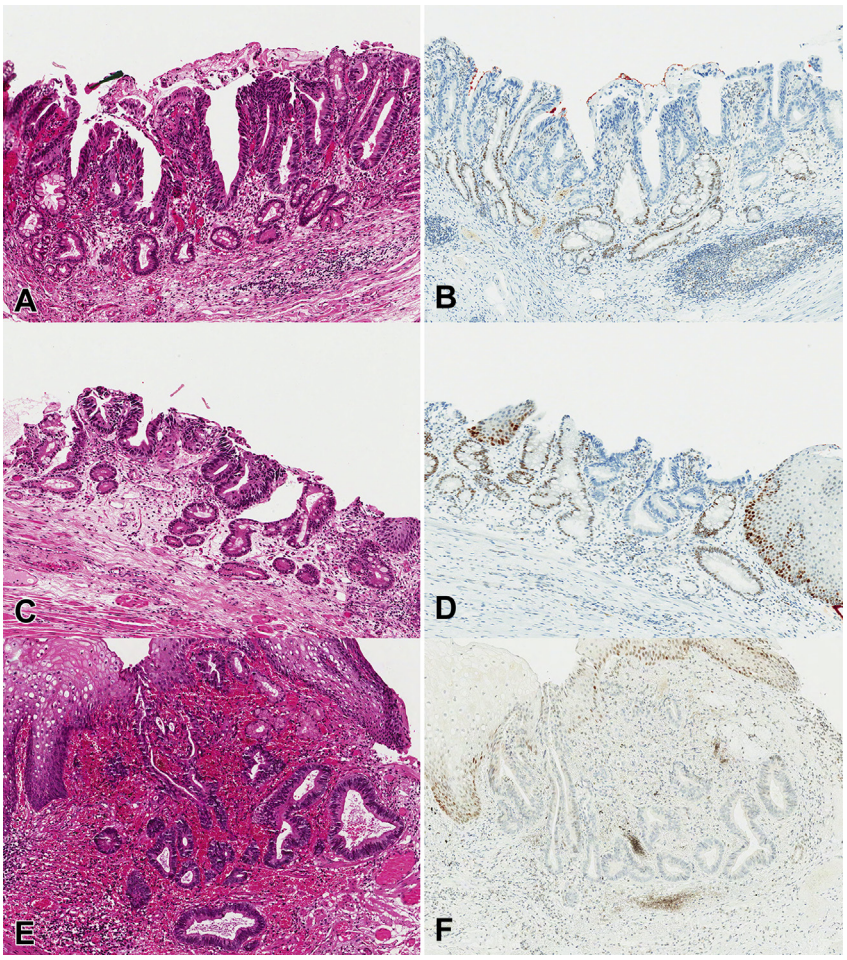

Fig. 7 (A–F) Loss of p53 expression in dysplastic Barrett's mucosa (pattern 4). Three cases of high grade dysplasia. Note the contrast with adjacent non-dysplastic glands and background stromal lymphocytes and basal keratinocytes (D,F). Contiguous intramucosal carcinoma also showed complete loss of p53 staining (E,F).

Table 1 Summary tabulation of the four categories of p53 expression observed in non-dysplastic and dysplasia/neoplastic Barrett's mucosa

Pattern	Seen in	Intensity of positive cells	Cell-to-cell intensity	Location of positive cells	Comment
Pattern 1: Wild-type p53 expression	Non-dysplastic glandular and squamous mucosa	Weak and focally moderate	Heterogeneous	Proliferation zone of crypts	Expanded proliferation zones of metaplastic epithelium can show increased staining
Pattern 2: Crypt predominant p53 overexpression	Low grade dysplasia	Moderate to strong	Homogeneous	Proliferation zone and base of crypts, with variable (focal to patchy) surface staining	More common in low grade compared to high grade dysplasia
Pattern 3: Diffuse p53 overexpression	Low and high grade dysplasia and contiguous intramucosal carcinoma	Moderate to strong	Homogeneous	Entire crypts and surface	More common in high grade dysplasia compared to low grade
Pattern 4: Loss of p53 expression (null phenotype)	Low and high grade dysplasia and contiguous intramucosal carcinoma	No staining in dysplastic/neoplastic cells	N/A	No staining in dysplastic/neoplastic cells in crypts and surface epithelium	Contrast with wild-type non-dysplastic/ non-neoplastic epithelium and background lymphocytes and keratinocytes

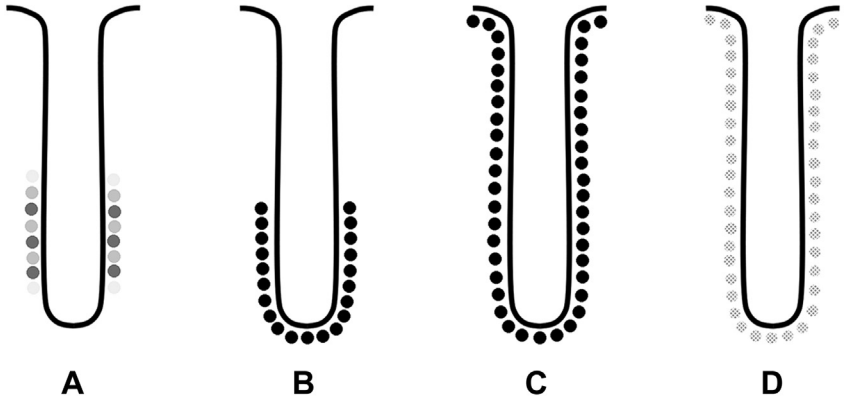

Fig. 8 Diagrammatic representation of patterns of p53 immunoreactivity. (A) Pattern 1, normal; (B) Pattern 2, over-expressed (crypt predominant); (C) Pattern 3, over-expressed (diffuse); (D) Pattern 4, loss of expression (null phenotype).

Conflicts of interest and sources of funding: The authors acknowledge funding and support of the Nuovo-Soldati Foundation for Cancer Research and state that there are no conflicts of interest to disclose.

Address for correspondence: Christopher Toon, Histopath, Suite 201, 4 Drake Avenue, Building B Pinnacle Office Park, North Ryde, NSW, 2113, Australia. E-mail: ctoon@med.usyd.edu.au

References

1. Edgren G, Adami HO, Weiderpass E, *et al*. A global assessment of the oesophageal adenocarcinoma epidemic. *Gut* 2013; 62: 1406–14.
2. Arnold M, Soerjomataram I, Ferlay J, *et al*. Global incidence of oesophageal cancer by histological subtype in 2012. *Gut* 2015; 64: 381–7.
3. Phoa KN, Pouw RE, Van Vilsteren FG, *et al*. Remission of Barrett's esophagus with early neoplasia 5 years after radiofrequency ablation with endoscopic resection: a Netherlands cohort study. *Gastroenterology* 2013; 145: 96–104.
4. Chadwick G, Groene O, Markar SR, *et al*. Systematic review comparing radiofrequency ablation and complete endoscopic resection in treating dysplastic Barrett's esophagus: a critical assessment of histologic outcomes and adverse events. *Gastrointest Endosc* 2014; 79: 718–31.
5. Downs-Kelly E, Mendelin JE, Bennett AE, *et al*. Poor interobserver agreement in the distinction of high-grade dysplasia and adenocarcinoma in pretreatment Barrett's esophagus biopsies. *Am J Gastroenterol* 2008; 103: 2333.
6. Whiteman DC, Appleyard M, Bahin FF, *et al*. Australian clinical practice guidelines for the diagnosis and management of Barrett's esophagus and early esophageal adenocarcinoma. *J Gastroenterol Hepatol* 2015; 30: 804–20.
7. Shaheen NJ, Falk GW, Iyer PG, *et al*. ACG clinical guideline: diagnosis and management of Barrett's esophagus. *Am J Gastroenterol* 2016; 111: 30.
8. Fitzgerald RC, di Pietro M, Ragunath K, *et al*. British Society of Gastroenterology guidelines on the diagnosis and management of Barrett's oesophagus. *Gut* 2014; 63: 7–42.
9. Bartek J, Bartkova J, Vojtěsek B, *et al*. Aberrant expression of the p53 oncoprotein is a common feature of a wide spectrum of human malignancies. *Oncogene* 1991; 6: 1699–703.
10. Goh AM, Coffill CR, Lane DP. The role of mutant p53 in human cancer. *J Pathol* 2011; 223: 116–26.
11. Prevo LJ, Sanchez CA, Galipeau PC, *et al*. p53-mutant clones and field effects in Barrett's esophagus. *Cancer Res* 1999; 59: 4784–7.
12. Contino G, Vaughan TL, Whiteman D, *et al*. The evolving genomic landscape of Barrett's esophagus and esophageal adenocarcinoma. *Gastroenterology* 2017; 153: 657–73.
13. Stachler MD, Camarda ND, Deitrick C, *et al*. Detection of mutations in Barrett's esophagus before progression to high-grade dysplasia or adenocarcinoma. *Gastroenterology* 2018; 155: 156–67.
14. Ten Kate FJ, Suzuki L, Dorssers LC, *et al*. Pattern of p53 protein expression is predictive for survival in chemoradiotherapy-naive esophageal adenocarcinoma. *Oncotarget* 2017; 8: 104123.
15. van der Wel MJ, Duits LC, Pouw RE, *et al*. Improved diagnostic stratification of digitised Barrett's oesophagus biopsies by p53 immunohistochemical staining. *Histopathology* 2018; 72: 1015–23.
16. Kaye PV, Ilyas M, Soomro I, *et al*. Dysplasia in Barrett's oesophagus: p53 immunostaining is more reproducible than haematoxylin and eosin diagnosis and improves overall reliability, while grading is poorly reproducible. *Histopathology* 2016; 69: 431–40.
17. Coleman H, van der Wel M, Jansen M, *et al*. PTH-118 Histopathologist features predictive of diagnostic concordance amongst an international sample of pathologists diagnosing barrett's dysplasia. *Gut* 2018; 67: A147.
18. Kaye PV, Haider SA, Ilyas M, *et al*. Barrett's dysplasia and the Vienna classification: reproducibility, prediction of progression and impact of consensus reporting and p53 immunohistochemistry. *Histopathology* 2009; 54: 699–712.
19. van der Wel M, Pouw RE, Seldenrijk KA, *et al*. Interobserver Agreement after addition of p53 staining within a digital expert panel for Barrett's esophagus. *Gastroenterology* 2017; 152: S448.
20. Lavery DL, Nicholson AM, Poulsom R, *et al*. The stem cell organisation, and the proliferative and gene expression profile of Barrett's epithelium, replicates pyloric-type gastric glands. *Gut* 2014; 63: 1854–63.
21. McDonald SA, Graham TA, Lavery DL, *et al*. The Barrett's gland in phenotype space. *Cell Mol Gastroenterol Hepatol* 2015; 1: 41–54.
22. Khan S, McDonald S, Wright N, *et al*. Crypt dysplasia in Barrett's oesophagus shows clonal identity between crypt and surface cells. *J Pathol* 2013; 231: 98–104.
23. Kastelein F, Biermann K, Steyerberg EW, *et al*. Aberrant p53 protein expression is associated with an increased risk of neoplastic progression in patients with Barrett's oesophagus. *Gut* 2013; 62: 1676–83.
24. Altaf K, Xiong J, Hickey L, *et al*. PTU-152 prediction of malignant progression of Barrett's oesophagus – a complete systematic review and meta-analysis. *Gut* 2016; 65: A132.
25. Kaye PV, Haider SA, James PD, *et al*. Novel staining pattern of p53 in Barrett's dysplasia – the absent pattern. *Histopathology* 2010; 57: 933–5.